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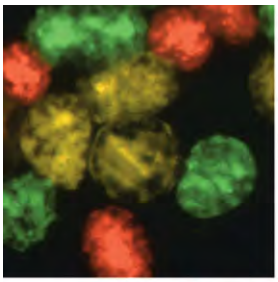

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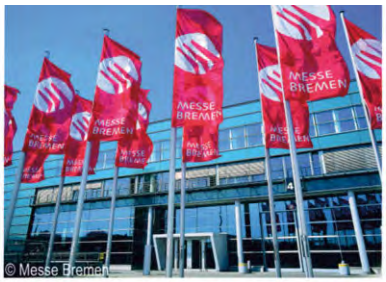

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2013
Sonderausgabe

Tagungsband zur

Jahrestagung der VAAM 2013 zusammen mit der KNVM

10.–13. März in Bremen

VAAM

Koninklijke Nederlandse Vereniging voor Microbiologie

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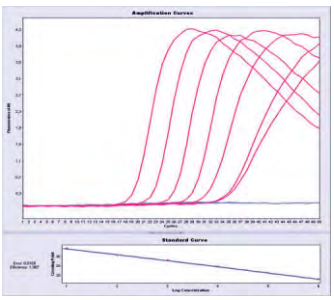
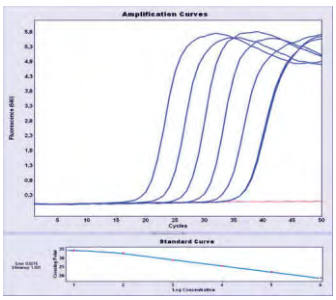


N. gonorrhoeae, dilution row 10 to 10E6 copies



M. genitalium, dilution row 10 to 10E6 copies

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Jahrestagung der Vereinigung für Allgemeine und Angewandte Mikrobiologie (VAAM) zusammen mit der Koninklijke Nederlandse Vereniging voor Microbiologie (KNVM)

Tagungsband zur Jahrestagung der VAAM 2013 zusammen mit der KNVM

10. bis 13. März in Bremen

Conference Co-Chairs: Rudolf Amann[6], Gert-Jan Euverink[8], Barbara Reinhold-Hurek[7], Matthias S. Ullrich[5]

Scientific Committee: Tilman Achstetter[3], Roland Benz[5], Jetta Bijlsma[8] , Karl-Heinz Blotevogel[7], Antje Boetius[1,6,7], Allan Cembella[1], Ulrich Fischer[7], Michael W. Friedrich[7], Frank Oliver Glöckner[5,6], Jens Harder[6], Jan Küver[2], Marcel Kuypers[6], Bernd Mahro[3], Katja Metfies[1], Georgi Muskhelishvili[5], Matthias Nagel[4], Manfred Schlösser[6], Marc Strous[6], Helge Weingart[5], Friedrich Widdel[6]

1: Alfred Wegener Institute, Bremerhaven
2: Bremen Institute for Materials Testing (MPA), Bremen
3: Hochschule Bremen, University of Applied Sciences, Bremen
4: Hochschule Bremerhaven, University of Applied Sciences, Bremerhaven
5: Jacobs University, Bremen
6: Max Planck Institute for Marine Microbiology, Bremen
7: University of Bremen, Bremen
8: University of Groningen, Groningen/NL

Zum Titelbild/Front Cover:

From left to right:
1st column: Fish image, underwater image Black Sea and genome circle (Max Planck Institute for Marine MIcrobiology)
2nd column: Messe Bremen, The Town Musicians (Fotolia), Bremen Market Place (Fotolia)
3rd column: Roland statue (Fotolia)

Liebe Teilnehmerinnen und Teilnehmer,

■ ich möchte Sie hiermit herzlich zur Jahrestagung der Vereinigung für Allgemeine und Angewandte Mikrobiologie begrüßen, die zusammen mit der Koninklijke Nederlandse Vereniging voor Microbiologie zum ersten Mal in Bremen ausgerichtet wird. Es freut mich sehr, dass sich mehr als 1400 Wissenschaftlerinnen und Wissenschaftler aus Deutschland, den Niederlanden und dem europäischen Ausland angemeldet haben, um sich hier auszutauschen und den Forschungsstandort Bremen noch besser kennen zu lernen. Mit zwei Universitäten, mehreren Hochschulen und zahlreichen Forschungsinstitutionen wie dem Alfred-Wegener-Institut für Polar- und Meeresforschung, dem Max-Planck-Institut für Marine Mikrobiologie, den Fraunhofer- und Leibniz-Instituten wird deutlich, dass Wissenschaft und Forschung in unserem Bundesland eine große Rolle spielen. Das zeigt sich auch an dem Erfolg bei der Exzellenzinitiative 2012.

Die Ergebnisse Ihrer Forschung, der Mikrobiologie, erlangen in einer wissensbasierten Gesellschaft eine immer größere Bedeutung. Hierbei denke ich nicht nur an die tragischen Fälle von EHEC-Infektionen und der Legionärskrankheit, die uns allen bekannt sind. Neben dem Wissen um pathogene Keime und Viren, die ja nur einen verschwindend geringen Bruchteil in der mikrobiellen Welt ausmachen, wird das Verständnis der Lebensweise von Mikroorganismen immer wichtiger. Viele traditionelle und auch moderne Bereiche des menschlichen Lebens werden von diesen Kleinstlebewesen dominiert, wie z.B. Lebensmittel- und Trinkwasserversorgung, Abwasserbehandlung, Bodensanierung, Abbau von Schadstoffen bis zu Klimafolgen. Die komplexe Bedeutung des mikrobiellen Lebens für ganze Ökosysteme steht teilweise noch ganz am Anfang ihrer Erforschung und zeigt die Notwendigkeit, sich mit diesen Themen wissenschaftlich auseinanderzusetzen.

Für diese Aufgaben und die Tagung wünsche ich Ihnen gutes Gelingen und einen angenehmen Aufenthalt in der Wissenschaftsstadt Bremen. ■

Jens Böhrnsen
Bürgermeister
Päsident des Senats der Freien Hansestadt Bremen

VAAM President's Greetings to the Annual Conference for 2013

■ Dear Colleagues and Friends,

This year's Annual Conference is a joint meeting of the *Association for General and Applied Microbiology (VAAM)* and the *Royal Netherlands Society for Microbiology (KNVM).* This represents a landmark of excellent cooperation and friendship between these two scientific organizations.

I am convinced that this conference will reinforce the relationship between VAAM and KNVM, as well as provide opportunities for individual scientific collaborations. In addition, we can expect an exciting program with contributions from members of both organizations. The organizers have selected a variety of interesting topics: Host-Microbe Interaction; Marine Microbiology; Physiology and Metabolism; Omics and Bioinformatics; Unicellular Eukaryotic Microbiology; Food and Feed Microbiology; Environmental Biotechnology; Single Cell Microbiology.

As this is a joint meeting together with KNVM, we expect an even higher number of participants than last year in Tübingen. There, we had close to 1,400 attendees. We can trust that the Bremen conference will continue the long series of highly attractive and successful annual VAAM conferences.

In addition to the standard program of mini-symposia from our "Fachgruppen" a first symposium will be held by VAAM's recently formed joint Biotransformation "Fachgruppe" and Dechema's Biocatalysis group. Every other year, this symposium will take place at the VAAM Annual Conference. We are planning to have a session on Industrial Microbiology when there is no "Fachgruppe Biotransformation" symposium. This session was well received last year in Tübingen and we want to continue to have a session on applied microbiology with speakers from industry.

I am very pleased to announce that Prof. Karl-Heinz Schleifer will become an honorary member of VAAM. The award ceremony will be held during the general meeting on March 12, 2013. Prof. Schleifer is an internationally renowned and distinguished researcher and has been a long-time ordinary member of VAAM. It is a great honor for VAAM to have Prof. Schleifer as an honorary member.

On behalf of the VAAM, I want to thank the conference chairs Rudolf Amann, Gert-Jan Euverink (KNVM), Barbara Reinhold-Hurek and Matthias Ullrich, as well as the local Scientific Committee, Conventus and Katrin Muth, for their great efforts and commitment in organizing the Annual Conference in Bremen. I also want to thank all scientists for their contributions. I am certain that we will have an exciting conference and stimulating discussions.

I would like to encourage you to join us in Bremen for this outstanding scientific event.

Hope to see you there.

Sincerely, ■

Gerhard Schmid

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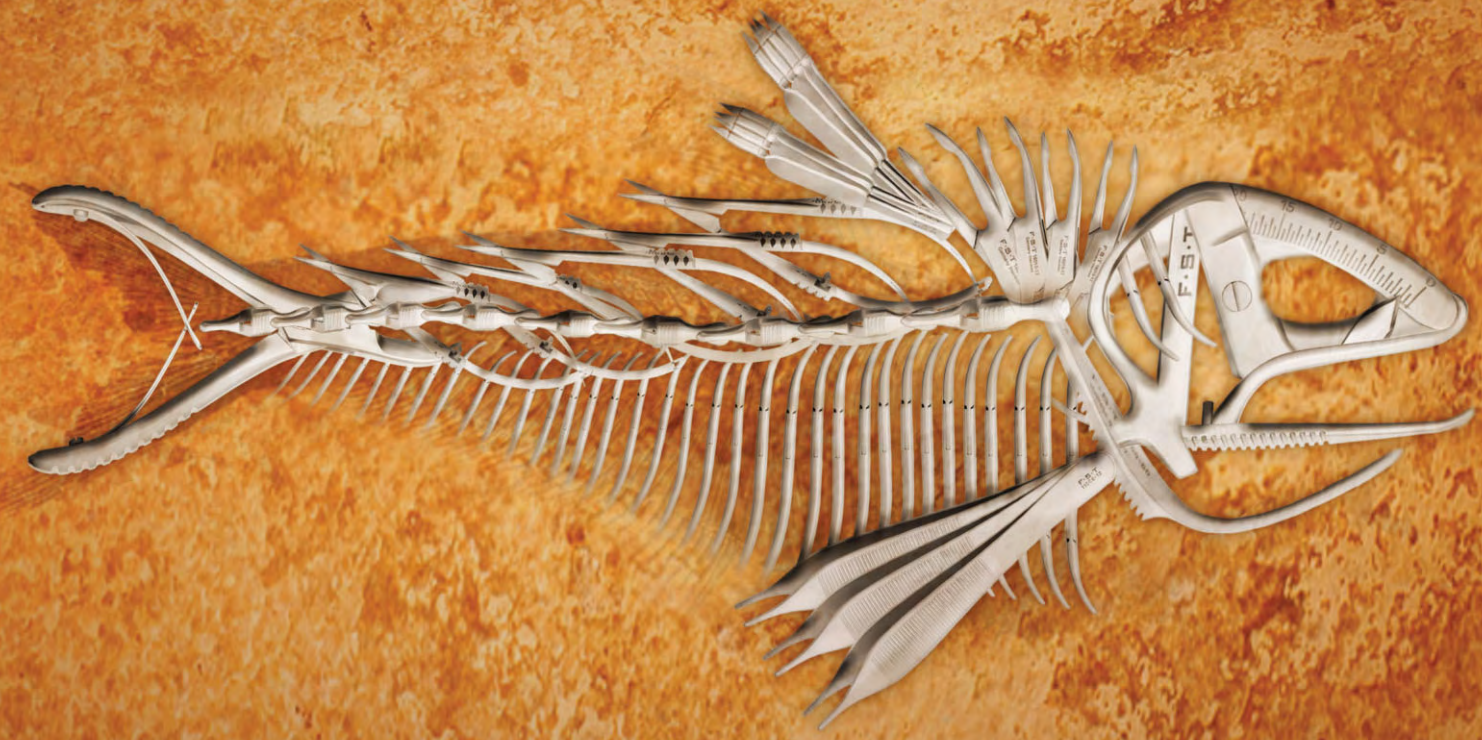

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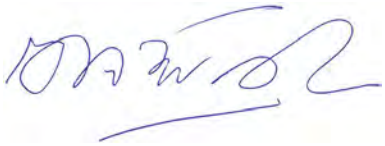
Greetings of the KNVM

Microbiology has been one of the leading fields of biology research in the Netherlands for more than a century. This is illustrated by the Dutch microbiologists Martinus Beijerinck, Albert Kluyver, Cornelis van Niel, Johanna Westerdijk and Christiaan Eijkman. Beijerinck and Westerdijk are well known for their pioneering work in virology and plant pathology, whereas Kluyver formulated concepts of unity in biochemistry. His pupil van Niel elaborated on the work of Kluyver and worked a long time in the United States at Stanford. There he organized a well known summer course with among its graduates Nobel Prize winners Arthur Kornberg (Chemistry of DNA synthesis), Konrad Bloch (1964, regulation of fatty acid and cholesterol metabolism), and Paul Berg (1980 recombinant DNA). Eijkman was the Nobel Prize winner in 1929 for his discovery of the vitamin (thiamine) deficiency in Beri-Beri. Recently, The Royal Dutch Academy of Arts and Sciences concluded that Microbiology was one of five biology research fields in the Netherlands that performs at the highest international level.

Dutch Microbiology is represented by the Royal Dutch Society for Microbiology KNVM. This society celebrated its centenary in 2011. Despite its age, KNVM is very vital. It has more than 1400 members, an all time record, with many young microbiologists that have taken up a function in the board of the society or in the boards of one of its sections. KNVM and the Dutch Society for Medical Microbiology organize each year a Spring Conference with about 750 participants. The ten sections of KNVM organize specialized autumn meetings that are attended by 40–100 participants. KNVM also focusses on education. For instance, the society publishes a book how to safely work with microorganisms that finds its way to schools, universities, research institutes, and companies each year. Moreover, KNVM organizes a bachelor master (BaMa) congress for microbiology students of Universities and Polytechnics.

KNVM was very delighted when VAAM informed whether the society would be interested to co-organize the 2013 meeting in Bremen. This meeting exposes Dutch and German microbiologists to a high quality program and offers a unique networking opportunity for young scientists and group leaders. KNVM welcomes all participants to the 2015 FEMS meeting in Maastricht to further intensify contacts between Dutch and German microbiologists.



Han Wösten
Chairman of the KNVM

Welcome Address of the Organizing Committee

It is our pleasure to welcome you in Bremen for the 2013 Conference of the Association for General and Applied Microbiology (VAAM) which is jointly organized with the Royal Netherlands Society for Microbiology (KNVM). We received more than 800 abstracts for posters and presentations and we tried to organize a program addressing not only many of the hot topics in microbiology, but also reserving time for the many special groups of VAAM and KNVM. All lectures will take place in the Congress Centrum Bremen near to the Bremen city center.

Bremen is a city with multiple faces – rich in history, with excellent science, and a strong industry encompassing cars, space and aviation, food, logistics as well as the harbors and offshore business. Together with its twin city Bremerhaven it forms the smallest of the federal states of Germany. Located on the river Weser the city of Bremen is home to 570,000 citizens. As a harbor city Bremen has always been open to the world and to the future. In combination with the century-old hanseatic traditions this makes for a particular Bremen flair. Distinctive landmarks are the magnificent Rathaus with its Weser Renaissance architecture and the Roland statue. In 2004 both have been designated UNESCO World Heritage sites. You will find a lively and multi-facetted collection of pubs and restaurants along the Schlachte, Bremen's entirely redesigned Weser promenade, but also in the old district Schnoor and in the "Viertel".

We hope that your stay in Bremen will be stimulating and exciting.

Rudolf Amann, Gert-Jan Euverink, Barbara Reinhold-Hurek, Matthias S. Ullrich, Tilman Achstetter, Roland Benz, Jetta Bijlsma, Karl-Heinz Blotevogel, Antje Boetius, Allan Cembella, Ulrich Fischer, Michael W. Friedrich, Frank Oliver Glöckner, Jens Harder, Jan Küver, Marcel Kuypers, Bernd Mahro, Katja Metfies, Georgi Muskhelishvili, Matthias Nagel, Manfred Schlösser, Marc Strous, Helge Weingart, Friedrich Widdel

Das Weinbuch für Mikrobiologen!

Helmut König / Heinz Decker (Hrsg.)

Kulturgut Rebe und Wein

Beim Wein handelt sich um ein Kulturgut, das im Abendland seit mehr als 2000 Jahren gepflegt wird. Jedoch lässt sich im Nahen und Fernen Osten die Weinbereitung aus der Kulturrebe *Vitis vinifera vinifera* mehr als 8000 Jahre zurückverfolgen. Trotz dieser langen Tradition und allgemeinen gesellschaftlichen Bedeutung sind die biochemischen und mikrobiellen Hintergründe, die zur Umwandlung von Most in Wein führen, erst vor etwa 150 Jahren durch den Chemiker Louis Pasteur bekannt geworden.

2013, 295 S. 126 Abb., 116 in Farbe. Geb.
ISBN 978-3-8274-2886-8
► € (D) 24,99 | € (A) 25,69 | *sFr 31,50

Im interdisziplinären Arbeitskreis „Rebe und Wein“ werden an der Johannes Gutenberg-Universität Mainz unterschiedliche Aspekte des Kulturgutes Wein untersucht. In diesem Sachbuch für Weininteressierte, das hervorgegangen ist aus der Vorlesungsreihe „Weinwissenschaft an der Universität Mainz“, fassen Fachautoren aus den Bereichen Mikrobiologie und Weinbereitung, Biophysik, Molekularbiologie, Chemie, Medizin, Sport, Literaturgeschichte, Religion, Pharmakologie, Psychologie, Sprachwissenschaften und Rechts- sowie Wirtschaftswissenschaften Grundlagen und neueste Forschungsergebnisse zum Thema **„Kulturgut Rebe und Wein“** zusammen. Damit wird das Thema in einer bisher noch nicht dagewesenen Breite beleuchtet.

General Information

Annual Conference 2013 of the Association for General and Applied Microbiology (VAAM) in collaboration with the Royal Netherlands Society for Microbiology (KNVM)

Venue

Congress Center Bremen (CCB) and
Messe Bremen
Theodor-Heuss-Allee 21–23
28215 Bremen (DE)
Germany

Address for correspondence

Conventus Congressmanagement &
Marketing GmbH
Isabelle Lärz
Carl-Pulfrich-Strasse 1
07745 Jena (DE)
Tel.: +49 (0)3641 311 63 20
Fax: +49 (0)3641 311 62 43
www.vaam-kongress.de

Opening hours

Sunday	10.03.2013	13:00 – 19:00
Monday	11.03.2013	08:00 – 19:30
Tuesday	12.03.2013	08:00 – 19:30
Wednesday	13.03.2013	08:30 – 13:00

Registration fees (all days)

VAAM-Members	
Regular	210 €
Student*	85 €
Industry Representatives**	300 €
Retiree	100 €
Non-members	
Regular	280 €
Student*	110 €
Industry Representatives**	370 €
Retiree	145 €
Fee for day tickets (Monday, Tuesday, Wednesday)	**100** €

* Please provide confirmation and quote VAAM 2013 as the reference.

** This fee is not for industry representatives taking part in the industrial exhibition. Special rates will be provided for booth personnel.

Social programme

Welcome reception*** (10 March 2013)	included
Mixer*** (12 March 2013)	included

*** Registration required.

Should you transfer your invoice amount within 10 days of the start of the event, please present your transfer remittance slip at the Check-In desk as proof of payment.

Travelling to Bremen

By train

From the main station it is a 3-minute walk to the Congress Center Bremen. There are hourly rail connections and nearly 50 ICE und IC/EC connections daily.

By public transport in Bremen

3 bus services get you to the exhibition centre easily: service 24 (to "Blumenthalstraße"), 26 and 27 (to "Messe Centrum").

If you want to use the tram, continue with line 6 or 8 to stop „Blumenthal Street".

For detailed directions on how to get to the CCB (or anywhere in the city), call +49 (0) 0421 – 59 60 59 or visit www.bsag.de.

By car

The Bremen Exhibition Centre and Congress Center Bremen is right in the heart of the city: next to the main railway station, only 10 minutes from the motorways (A1, A27, A28) exit to the exhibition car park or the open-air site with 2,500 parking bay. From the motorway exits simply follow the signs to Centrum/ Messe Bremen.

It is also located in the direct vicinity of numerous hotels, restaurants and the expansive Bürgerpark.

Car park

For the car park "Bürgerweide" please enter "Theodor-Heuss-Allee, 28215 Bremen" into your navigation system.

For the multi-storey car park please enter "Hollerallee 99, 28215 Bremen" into your navigation system.

By plane

Bremen has its own City Airport. From the airport, you can take tram 6 (travels every 10 minutes) towards the city centre (Stadtzentrum/Hauptbahnhof). The Congress Centrum can be reached from station "Blumenthalstraße". The Congress Centrum is approx. 3 minute walk from the station.

Distance from Congress Centrum Bremen: 7 km

Taxi costs from airport: ca. 15 € (Taxis drive up in front of the CCB or taxi stand at the northern exit of the main station.)

Hotel reservation

We have reserved contingents in selected hotels in cooperation with HRS (Hotel Reservation Service).

Please visit www.vaam-kongress.de/hotels.

Registration and conference fees

Online registration is possible till 07 March 2013 on the conference homepage at www.vaam-kongress.de. Registration after this date is possible on-site only. Beside cash payments we also accept credit cards at the conference Check-In desk (Master/ Euro, VISA, American Express and JBC) as well as EC-Cards.

Mixer

The Mixer will take place on Tuesday, 12 March 2013 at 19:30 in the Weserstadion Bremen. Accompanying persons may purchase

a ticket for the mixer at the conference Check-In desk.

How to get there

From the Central Station: Take tram line 10 in the direction of "Sebaldsbrück" and get off at the stop "St.-Jürgen-Straße".

From "Domsheide": Take tram line 2 in the direction of "Sebaldsbrück" and get off at the stop "St.-Jürgen-Straße" **OR** take tram line 3 in the direction of "Weserwehr" and get off at the stop "Weser-Stadion".

For further information (e.g. walking maps), please refer to the Check-In desk on-site.

Posters

Posters are divided according to number into the two poster sessions. All odd poster numbers are in the poster session on Monday, 11 March 2013, 15:15–17:30. Even poster numbers are in the poster session on Tuesday, 12 March 2013, 15:30–17:30.

Posters are to be presented in English and in the format DIN A0 (84.1 cm × 118.9 cm) and no lamination. Authors are asked to attach to the posters the time when they will be available for discussion. The posters will have to be fixed by pins. Materials will be provided.

The posters may be hung from 14:00 on Sunday, 10 March 2013 and should be removed before 12:00 on Wednesday, 13 March 2013.

Presentation of the Honorary Award, PhD Awards, and Poster Prizes

The presentation of the Honorary Award will take place on 11 March 2013 at 11:00.

The presentation of the PhD Thesis prizes will take place on 12 March 2013 at 18:15.

The presentation of the poster prizes will take place on 13 March 2013 at 11:30.

The VAAM Honorary Award and the Poster Prizes will be presented in the "Hanse-Saal".

The PhD Thesis prizes will be presented in the "Kaisen-Saal".

Short lectures

The length of short lectures has been fixed to a max. of 10 minutes plus 5 minutes for discussion (some variation may apply). Due to the fact that there will be up to 7–8 parallel sessions please adhere to the total time allotted to you.

Short lectures are to be held in English. Data projectors are available in each of the lecture halls. In each lecture hall there will be an assistant for technical support. We ask all lecturers to make use of the computer facilities located at our **presentation submission** to check their presentations in advance.

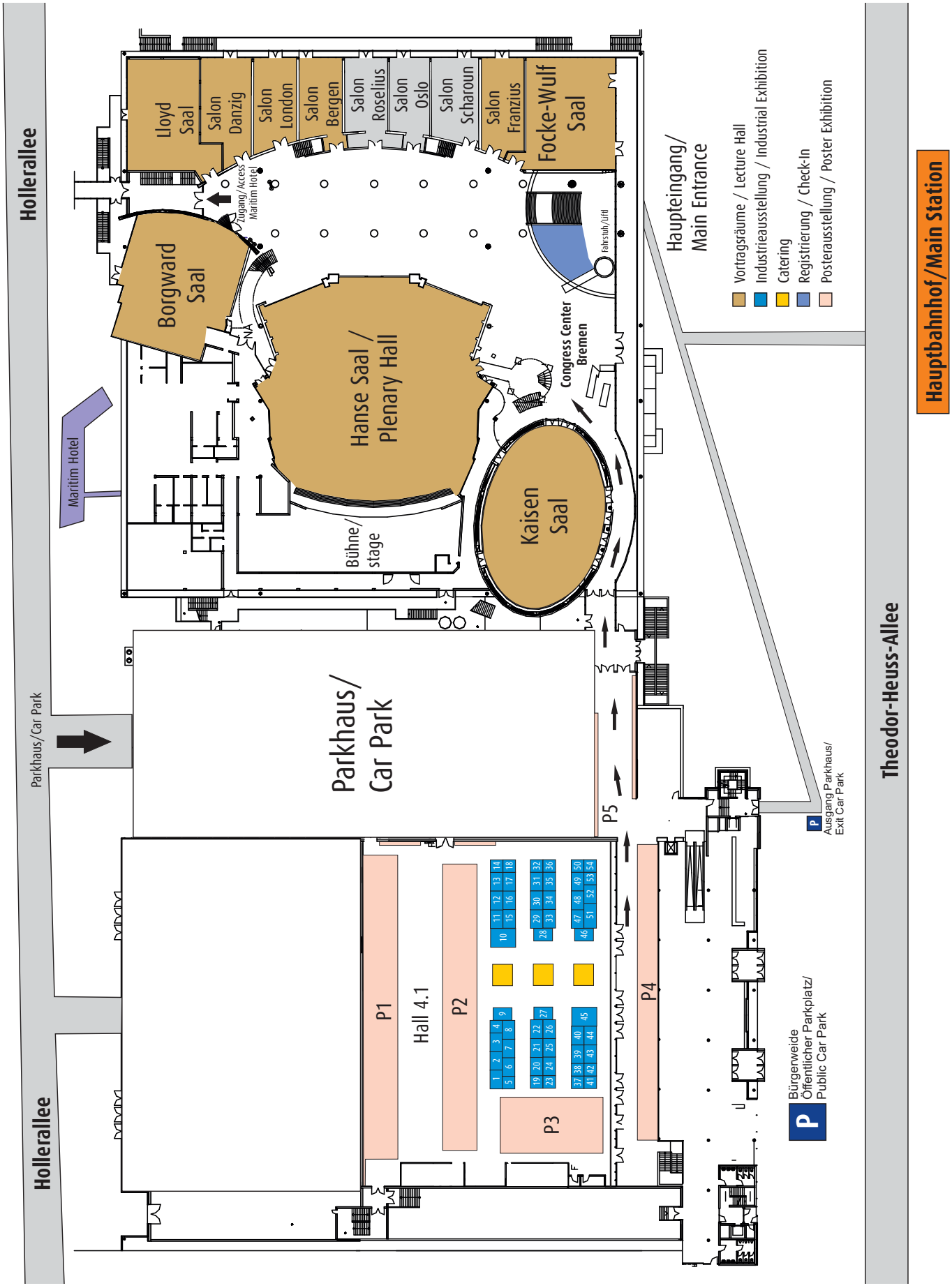
General Tips for Authors and Presenters

Presentation Submission

Please hand in your presentation of your talk in the Presentation Submission room. Follow the signage on-site or ask at the Check-In desk.

Time Allotment

To ensure smooth running of the entire programme, all speakers are encouraged to adhere to their allocated speaking time. The chair persons of the sessions are urged to cancel discussions in delay. Contact your chair per-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Zugang/Access Maritim Hotel
Borgward Saal
Maritim Hotel
Hanse Saal / Plenary Hall
Congress Center Bremen
Fahrstuhl/Lift
Bühne/stage
Kaisen Saal
Haupteingang/Main Entrance
Vortragsräume / Lecture Hall
Industrieausstellung / Industrial Exhibition
Catering
Registrierung / Check-In
Posterausstellung / Poster Exhibition
Parkhaus/Car Park
P5
Ausgang Parkhaus/Exit Car Park
Theodor-Heuss-Allee
Hauptbahnhof/Main Station
P1
Hall 4.1
P2
P3
P4
Bürgerweide
Öffentlicher Parkplatz/Public Car Park

son before your session begins and advise of any changes or special wishes.

Presentation Form and Submission of Presentation

Please submit your presentation at least 120 minutes before your lecture will start. You are asked to clearly label your CD/memory stick and the file with your short lecture code number and the name of the person giving the talk. All presentations will be loaded onto our computers and will be deleted after the talks.

PDF and PowerPoint presentations are permitted. Open Office formats may also be used. Required technical equipment will be available at the congress.

The use of Macintosh or Open Office formats as well as the use of a personal laptop for a presentation is not planned, but possible. If necessary, please contact us by 15 February 2013 at vaam-kongress@conventus.de.

For video and audio files please submit AVI, WMV and MPG files only as a separate file.

Please make sure that any required CODEC files for any videos are also submitted.

Your presentation and any additional files should be submitted at least two hours before your presentation time.

Please note: If you use a USB stick to save your files, do not protect it with software.

Karrieresymposium

Vorstellung vielfältiger Berufsbilder in den Biowissenschaften
Anregungen und Tipps

Montag, 11. 3. 2013

15.30 – 17.00 Uhr
Kaisen-Saal

Tobias Erb, Zürich
Junior- Gruppenleiter ETH Zürich
Vom Doktoranden zum Gruppenleiter – Ein Erfahrungsbericht aus drei Ländern

Jan Küver, Bremen
Abteilungsleiter Materialprüfungsanstalt Bremen
Als Mikrobiologe in die Materialwissenschaften – Forschen und Arbeiten bei der Materialprüfungsanstalt

Guido Ketschau, Bremen
Unternehmensverband LifeSciences Bremen; Ketschau Consulting
Vom Wissenschaftler zum Unternehmer – Facetten der Existenzgründung im Spannungsfeld Wissenschaft und Markt

Einladung zur Mitgliederversammlung der VAAM

■ Hiermit lade ich alle Mitglieder der VAAM zur Mitgliederversammlung ein. Sie wird am Dienstag, den 12. März 2013, um 17.00 Uhr im Kaisen Saal im Congress Center in Bremen stattfinden.

Vorläufige Tagesordnung:

1. Festlegung der Tagesordnung und Genehmigung der Niederschrift der Mitgliederversammlung vom 21. März 2012 in Tübingen (siehe BIOspektrum 3/12, Seiten 308 und 309)
2. Bericht aus dem Vorstand, u.a. Haushalt 2012 und Haushaltsplan 2013, Ort und Zeit der nächsten Jahrestagung, Aktivitäten der Fachgruppen, VBIO, DGHM
3. Bericht der Kassenprüfer
4. Entlastung des Vorstandes
5. Änderung der Satzung (Zweck, studentische Mitglieder) und der Geschäftsordnung (*online*-Wahl der Ehrenmitglieder), siehe Kasten
6. Mikroorganismus des Jahres
7. Wahl des Präsidiums (Präsident, 1. Vizepräsident, Schatzmeister, Schriftführer) und drei der sechs Mitglieder des Beirates (geheime Wahl während der Mitgliederversammlung)
8. Wahl eines Ehrenmitglieds
9. Verschiedenes

Im Anschluss:
- Verleihung der Ehrenmitgliedschaft an Karl-Heinz Schleifer
- Verleihung der VAAM-Promotionspreise 2013

Hiermit bitte ich alle Mitglieder, Vorschläge zur Wahl des Präsidiums und des Beirates beim Präsidenten einzureichen (bis 14 Tage vor der Mitgliederversammlung), wobei Vorschläge für das Präsidium von zehn VAAM-Mitgliedern und für den Beirat von drei Mitgliedern unterschrieben sein müssen. Ich möchte auch darauf hinweisen, dass der Vorstand der VAAM den jetzigen 1. Vizepräsidenten entsprechend der Geschäftsordnung (siehe Homepage der VAAM) zur Wahl zum Präsidenten vorschlagen wird. Ordentliche und studentische Mitglieder haben auf der Mitgliederversammlung gleiches Stimmrecht.

Reisekostenzuschüsse für studentische Mitglieder können bei fristgerecht eingegangenen Anträgen und bei Vorliegen der sonstigen Voraussetzungen nur persönlich am **Dienstag, den 12. März 2013 zwischen 14.00 und 16.00 Uhr** und am **Mittwoch, den 13. März 2013 von 10.00 – 12.00 Uhr** im Tagungsbüro abgeholt werden. ■

Hubert Bahl
Schriftführer

Satzungsänderung

Neuerungen sind farblich hervorgehoben

Alt:
§ 1 Name, Sitz, Zweck
2. Die Vereinigung hat die Aufgabe, die wissenschaftliche Kommunikation unter den in Forschung, Lehre und Praxis tätigen Mikrobiologen und interessierten Wissenschaftlern der Nachbardisziplinen zu intensivieren sowie die Ausbildung junger Berufskollegen zu fördern. Jährlich wird mindestens eine Tagung mit wissenschaftlichem Programm abgehalten.

Neu:
§ 1 Name, Sitz, Zweck
2. Die Vereinigung hat die Aufgabe, die wissenschaftliche Kommunikation unter den in Forschung, Lehre und Praxis tätigen Mikrobiologen und interessierten Wissenschaftlern der Nachbardisziplinen zu intensivieren sowie die Ausbildung junger Berufskollegen und -kolleginnen zu fördern, insbesondere durch die Verleihung von Promotions- und Forschungspreisen. Jährlich wird mindestens eine Tagung mit wissenschaftlichem Programm abgehalten, bei der die aktive Teilnahme von Studierenden durch Reisebeihilfen unterstützt wird.

Alt:
§ 2 Mitglieder
3. Als studentische Mitglieder können aufgenommen werden: Studierende der Biologie und der Nachbardisziplinen, die das Vorexamen bzw. ein Bakkalaureat bestanden haben, sowie auf begründetem Antrag auch technische Mitarbeiter im Fachgebiet Mikrobiologie, die sich in einer Weiterbildungsphase befinden. Der Antrag muss von einem ordentlichen Mitglied der VAAM unterstützt sein. Über die Aufnahme entscheidet eine vom Präsidium benannte sachkundige Person.

Neu:
§ 2 Mitglieder
3. Als studentische Mitglieder können aufgenommen werden: Studierende der Biologie und der Nachbardisziplinen sowie auf begründeten Antrag auch technische Mitarbeiter/innen im Fachgebiet Mikrobiologie, die sich in einer Weiterbildungsphase befinden. Über die Aufnahme entscheidet eine vom Präsidium benannte sachkundige Person.

Änderung der Geschäftsordnung

Alt:
III. Ehrenmitgliedschaft
Personen, die die Gesellschaft und ihren Wirkungsbereich in hervorragender Weise gefördert haben, können vom Vorstand auf einstimmigen Beschluß zu Ehrenmitgliedern vorgeschlagen werden. Die Mitgliederversammlung beschließt über diesen Vorschlag ohne Diskussion mit 2/3-Mehrheit der anwesenden Mitglieder. Ehrenmitglieder haben alle Rechte der ordentlichen Mitglieder, sind aber von der Zahlung von Mitglieds und Tagungsbeiträgen befreit.

Neu:
III. Ehrenmitgliedschaft
Personen, die die Gesellschaft und ihren Wirkungsbereich in hervorragender Weise gefördert haben, können vom Vorstand auf einstimmigen Beschluss zu Ehrenmitgliedern vorgeschlagen werden. Die Mitglieder entscheiden über diesen Vorschlag *online* mit einfacher Mehrheit. Ehrenmitglieder haben alle Rechte der ordentlichen Mitglieder, sind aber von der Zahlung von Mitglieds- und Tagungsbeiträgen befreit.

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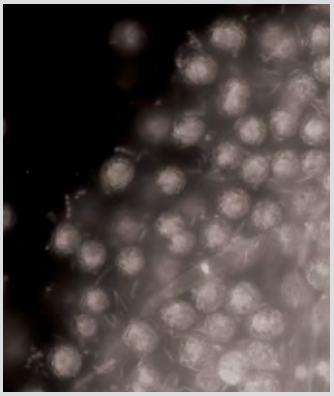
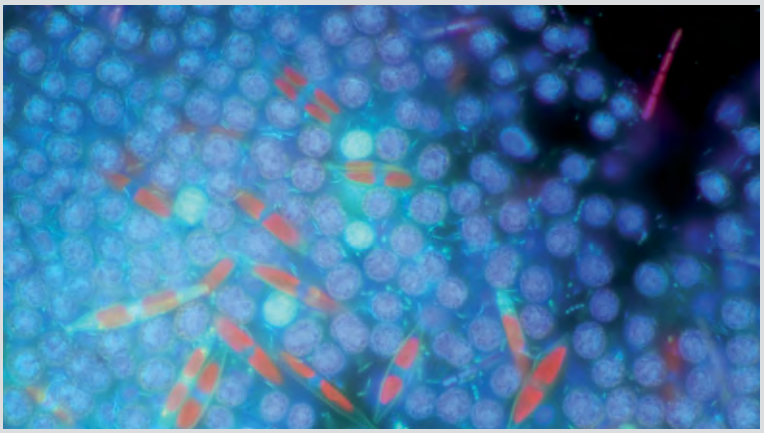
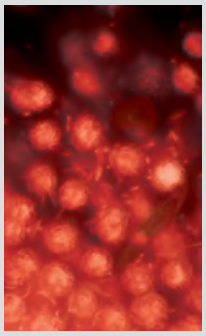

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65. Jahrestagung der Deutschen Gesellschaft für Hygiene und Mikrobiologie (DGHM) e. V.

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Fachgruppe Archaea

Archaea sind mittlerweile als dritte Domäne des Lebens im Bewusstsein der Mikrobiologen fest verankert. Zu ihrer besonderen Beachtung haben nicht nur ihr häufiges Vorkommen an extremen Standorten (heiß, sauer, alkalisch oder salzig) beigetragen, sondern auch weitere Besonderheiten in ihrer Physiologie oder ihren Zellstrukturen. Archaea kommen fast ubiquitär auf diesem Planeten vor, was die Bedeutung dieser Mikroorganismen unterstreicht. Über genetische Nachweismethoden wurden Archaea in Boden und Wasserhabitaten entdeckt, allerdings sind viele davon noch nicht kultiviert.

Mitglieder der Fachgruppe beschäftigen sich mit einem breiten Spektrum an Untersuchungen zur Ökologie, Physiologie, Molekularbiologie und den Zellstrukturen von Archaea. Dabei lassen immer wieder neue Erkenntnisse und spannende Entdeckungen aufhorchen, die auch dazu führen, gängige Konzepte zu hinterfragen. So zeigt die Auswertung von Metagenomanalysen, dass Archaea am N-Kreislauf stärker beteiligt sind als bislang vermutet. Auch die Lebensgemeinschaft *Ignicoccus – Nanoarchaeum* hat Besonderheiten bei der Lokalisation von Enzymen in der Zelle zu Tage gefördert, da die Energie liefernden Enzyme in der „äußeren" Membran von *Ignicoccus* lokalisiert sind. Auch wegen ihrer phylogenetischen Stellung sind Archaea im Hinblick auf die Evolution von Enzymen spannende Untersuchungsobjekte. Neue Forschungen zeigen, dass viele Archaea in der Lage sind, an Oberflächen zu binden und Biofilme auszubilden. Da einige Archaea relativ leicht genetisch manipulierbar sind, können auch funktionelle Analysen durchgeführt werden. Allerdings müssen Untersuchungsmethoden für die Forschung an hyperthermophilen und extrem halophilen Archaea häufig erst adaptiert werden.

Die Fachgruppe Archaea bildet ein Forum, in dem neue Erkenntnisse diskutiert und auch Erfahrungen bei der Entwicklung von Untersuchungsmethoden ausgetauscht werden. Höhepunkt 2012 war die von Sonja-Verena Albers, Finn Werner und Bettina Siebert organisierte Tagung „Molecular Biology of Archaea" (2.–4. Juli am MPI in Marburg), wo hervorragende internationale Arbeiten vorgetragen wurden. Ansonsten trifft sich die Fachgruppe jährlich im Herbst auf einer von Jörg Soppa organisierten Tagung in Schmitten bei Frankfurt, bei der vor allem Doktoranden ihre Daten zur Diskussion stellen und internationale Sprecher zum Vortrag eingeladen werden.

Sprecherin: Felicitas Pfeifer, Universität Darmstadt, Email: pfeifer@bio.tu-darmstadt.de

Fachgruppe Biologie bakterieller Naturstoffproduzenten

Die Fachgruppe „Biologie bakterieller Naturstoffproduzenten" beschäftigt sich mit vielfältigen Aspekten der Naturstoffbiosynthese. Dazu gehören genetische Grundlagen der Sekundärmetabolitproduktion genauso wie deren Regulation und Biochemie. Die Fachgruppe war ursprünglich auf Streptomyceten fokussiert, die eine bedeutende Rolle als mikrobielle Antibiotikaproduzenten spielen. Neben den Naturstoffbiosynthesen waren von Beginn an auch charakteristische biologische Aspekte dieser Bakteriengruppe wie Zelldifferenzierung, Synthese von Exoenzymen und Genomstruktur Schwerpunktthemen der Fachgruppe.

Da sich aber sowohl bei den Biosyntheseleistungen als auch bei der Zelldifferenzierung und Biologie von Actinomyceten einige Parallelen zu anderen Naturstoffproduzenten wie Myxobakterien und Cyanobakterien zeigten, wurde das Konzept der Fachgruppe erweitert. Die Fachgruppe bietet nun allen VAAM-Mitgliedern, die ein Interesse an Naturstoffbiosynthesen sowie der Biologie bakterieller Naturstoffproduzenten haben, einen fachlichen Rahmen. Die Fachgruppe ist nicht zuletzt deshalb auch stark interdisziplinär ausgerichtet und vereint neben Kollegen der Mikrobiologie auch solche der Chemie und Pharmazie. Die insgesamt rund 200 Mitglieder widmen sich Themen der Grundlagenforschung genauso wie angewandten Aspekten. Traditionell gibt es eine enge Verbindung zur Pharmazeutischen Industrie, wo vor allem das therapeutische Potenzial der Naturstoffproduzenten von Interesse ist.

Die Fachgruppe beteiligt sich an der Organisation internationaler Tagungen (wie der „1. European Conference on Natural Products" im September 2013 in Frankfurt) und veranstaltet seit 1985 jährlich einen Workshop, auf dem vor allem jungen Mitgliedern die Gelegenheit gegeben wird, ihre Forschungsergebnisse zu präsentieren. Das nächste Treffen wird voraussichtlich im September 2013 in Frankfurt stattfinden.

Sprecher: Elke Dittmann, Universität Potsdam Email: editt@uni-potsdam.de

Stellvertretender Sprecher: Wolfgang Wohlleben, Universität Tübingen, Email: wolfgang.wohlleben@biotech.uni-tuebingen.de

Fachgruppe Fungal Biology and Biotechnology/Experimentelle Mykologie

■ Unserem wissenschaftlichen Nachwuchs, also Doktoranden, Post-Docs und Habilitanden, zweimal im Jahr ein Forum zur Diskussion eigener Ergebnisse zu bieten, ist das Ziel unserer Fachgruppe. In der Regel wird mindestens ein besonders angesehener ausländischer Gastsprecher eingeladen.

Auch im Jahr 2012 konnten wir helfen, dieses Ziel zu erreichen. Wir danken Vera Meyer (Berlin) und Sven Krappmann (Würzburg) für die Organisation eines sehr gut besuchten Minisymposiums im Rahmen der Frühjahrstagung in Tübingen. Zweifellos ein Höhepunkt für alle Pilz-Begeisterten war die von Regine Kahmann in Marburg veranstaltete „11th European Conference on Fungal Genetics – ECFG 11".

Im Jahr 2013 wird sich die Unterstützung durch die Fachgruppe auf eine von Reinhard Fischer in Karlsruhe geplante Tagung konzentrieren. Vom 29. September bis 3. Oktober 2013 wird dort die „XI International Conference on Fungal Biology" stattfinden. Diese Tagung fand zuletzt 2009 in Mexico statt. Die in Deutschland im zweijährigen Rhythmus etablierte Tagung „Molekularbiologie der Pilze" wird nicht separat stattfinden, sondern soll inhaltlich integriert werden. ■

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Stellvertetende Sprecherin:
Ursel Kües,
Universität Göttingen
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Fachgruppe Symbiotische Interaktionen

■ Die derzeit jüngste VAAM-Fachgruppe (gegr. Dezember 2009) umfasst aktuell rund 90 Mitglieder aus Universitäten, Forschungseinrichtungen und der Industrie. Ziel unserer Fachgruppe ist es, den regelmäßigen Kontakt und Austausch zwischen den Arbeitsgruppen zu fördern, die Arbeiten der Fachgruppe international sichtbar zu machen sowie gemeinsame Fortbildungsveranstaltungen für den wissenschaftlichen Nachwuchs durchzuführen. Die Forschungsaktivitäten sind, ebenso wie die Fachgruppe selbst, stark interdisziplinär ausgerichtet. Im Vordergrund stehen die vielfältigen Interaktionen von Mikroorganismen mit tierischen, menschlichen oder pflanzlichen Wirten.

Die Fachgruppe richtete auf der vergangenen Jahrestagung 2012 in Tübingen ein Minisymposium mit dem Titel „Symbiotic Interactions" aus. Im Vordergrund standen ausgewählte Aspekte der Symbioseforschung, wie beispielsweise Diversität, Stoffwechsel, Evolution und Symbiosefaktoren. Den Einführungsvortrag hielt Gastsprecherin Virginia Weis (Corvallis, OR, USA) zum Thema Korallensymbiosen. Insgesamt spiegelte diese sehr gut besuchte Veranstaltung die ganze Bandbreite der Symbioseforschung in Deutschland wider. Neben wissenschaftlichen Inhalten wurde eine Mitgliederversammlung abgehalten, bei der Ute Hentschel Humeida (Würzburg) und Andreas Schwiertz (Herborn) einstimmig als Sprecher der Fachgruppe wiedergewählt wurden.

Auf der VAAM-Jahrestagung 2013 in Bremen wird unsere Fachgruppe ein eigenes Minisymposium mit dem Titel „Microbial Ecology of Mice and Man" veranstalten, um dem aktuellen Forschungsstand auf dem Gebiet der menschlichen Mikrobiota Rechnung zu tragen. Da diese Jahrestagung in Kooperation mit den niederländischen Mikrobiologen stattfindet, freut es uns besonders, dass es uns gelungen ist, als Gastredner zwei Sprecher zu gewinnen, die alle drei Bereiche (Deutschland, Niederlande, Mice and Men) in ihrer Arbeit vereinen: Hauke Smidt, Laboratory of Microbiology, Wageningen University, Niederlande: „Monogastric Model Animals – Untangling the Interplay of Microbiota with the Development of its Mammalian Host" und Vitor Martins dos Santos, Chair for Systems and Synthetic Biology, Wageningen University, Niederlande: „Systems approaches to understand the interplay between gut microbiota and its mammalian host". Weitere interessante Vorträge im Rahmen unseres Minisymposiums werden diverse Aspekte der Interaktion von Mikroorganismen mit ihren Wirten präsentieren. ■

Sprecherin:
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Stellvertetender Sprecher:
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Internet: http://www.helmholtz-muenchen.de/en/symbiotic-interactions/home-aims/index.html

Fachgruppe Lebensmittelmikrobiologie

■ Nach der gemeinsamen Tagung der Fachgruppe Lebensmittelmikrobiologie der VAAM und DGHM vom 28. bis 30. März 2012 in Stuttgart-Hohenheim haben keine weiteren Aktivitäten der Fachgruppe der VAAM stattgefunden.

Das 14. Fachsymposium „Lebensmittelmikrobiologie“ findet erneut als gemeinsame Veranstaltung der VAAM- und DGHM-Fachgruppen Lebensmittelmikrobiologie vom 22. bis 24. April 2013 in der Evangelischen Akademie Tutzing am Starnberger See statt. Keynote Speaker wird Prof. Dr. Douwe van Sinderen (University College Cork, Irland) mit einem Vortrag zu „Bakteriophagen“ sein. In der Mitgliederversammlung während des Symposiums wird ein neuer Sprecher der Fachgruppe gewählt, da der jetzige Sprecher – Knut J. Heller, Max-Rubner-Institut Kiel – aus Altersgründen ausscheiden wird. ■

Sprecher: Knut J. Heller, Max-Rubner-Institut Kiel
Email: knut.heller@mri.bund.de

Stellvertretender Sprecher: Siegfried Scherer, TU München
Email: Siegfried.Scherer@wzw.tum.de

Fachgruppe Hefe

■ Während der diesjährigen VAAM-Jahrestagung in Tübingen fand am ein Symposium der Fachgruppe Hefe statt. Acht Vortragsthemen umfassten die Bereiche Membranen und Endozytose, RNA und Ribosomen sowie Biotechnologie. Die Veranstaltung fand regen Zuspruch und die Vorträge wurden intensiv diskutiert.

Vom 29. August bis 3. September 2013 wird sich die Fachgruppe an der Internationalen Konferenz „Yeast Genetics and Molecular Biology“ beteiligen, die in Frankfurt stattfindet. Zu dieser Tagung werden rund 1.000 Teilnehmer erwartet. Die Homepage der Konferenz wird im Mai zugänglich sein (www.yeast-2013.org). Die VAAM wird diese Tagung bei der Rednerfinanzierung unterstützen. Aufgrund der geplanten großen internationalen Hefetagung wird die Fachgruppe Hefe bei der VAAM-Tagung in Bremen kein Fachgruppen-Symposium durchführen. ■

Sprecher: Karl-Dieter Entian, Universität Frankfurt
Email: entian@bio.uni-frankfurt.de

Fachgruppe Identifizierung und Systematik

■ Wie fast jedes Jahr wurde auch während der VAAM-Jahrestagung 2012 in Tübingen wieder ein Minisymposium veranstaltet mit dem Topic „Quo vadis?“ Dazu gab es je einen Vortrag von Ian. C. Sutcliffe und Brian J. Tindall mit den Titeln „The road ahead for microbial systematics: raising our game in the postgenomic era“ und „The purpose of prokaryote systematics; clarifying muddy waters“. Zusätzlich wurden zwei Kurzvorträge zu den Themen „Phylogenie von Vakuolen-bildenden Schwefelbakterien“ und „Phylogenie der Chytridiomycota und Zygomycota“ präsentiert. Diese Veranstaltung war erfreulich gut besucht.

In der nachfolgenden Versammlung der Fachgruppenmitglieder wurde Hans-Jürgen Busse zum neuen Sprecher der Fachgruppe Identifizierung und Systematik gewählt und Brian Tindall zum Stellvertreter.

Im Sommer wurde eine Umfrage an die Mitglieder der Fachgruppe ausgesandt. Von mehr als 160 Mitgliedern kam aber nur von 11 Mitgliedern eine Rückmeldung; weitere 22 Adressaten der Umfrage waren mit der bei der VAAM-Geschäftsstelle hinterlegten Adresse nicht zu erreichen und zwei gaben ihr Ausscheiden aus der Fachgruppe bekannt. Erfreuliches Ergebnis der Umfrage war, dass niemand eine Auflösung der Fachgruppe befürwortet (die stille Zustimmung der nicht antwortenden Mitglieder vorausgesetzt). Auch sind einige Mitglieder dazu bereit, stärker an den Aktivitäten der Fachgruppe mitzuwirken. Die Art und Weise dieser Mitarbeit muss nun diskutiert werden. ■

Sprecher: Hans-Jürgen Busse, Universität Wien
Email: Hans-Juergen.Busse@vetmeduni.ac.at

Stellvertretender Sprecher: Brian Tindall, DSMZ Braunschweig
Email: bti@dsmz.de

Fachgruppe Qualitätssicherung und Diagnostik

■ Auf der Frühjahrstagung der VAAM in Tübingen im März 2012 organisierte die Fachgruppe Qualitätssicherung und Diagnostik zwei Aktivitäten: Bei einem Minisymposium stand diesmal anstelle von Fachpräsentationen im Vordergrund, Bachelor- und Masterabsolventen eine Perspektive aufzuzeigen, welche vielfältigen beruflichen Möglichkeiten sich im Umfeld der Qualitätssicherung und Diagnostik ergeben können. Vier Mitglieder der Fachgruppe schilderten ihren Karriereweg und ihre beruflichen Erfahrungen.

Dieses Minisymposium war mit rund 150 Teilnehmern bestens besucht. Zwischen Zuhörern und Vortragenden entstanden rege Diskussionen. Dies zeigt, welcher Informationsbedarf gerade seitens der jüngeren Teilnehmer der Frühjahrstagung zu diesen Themen besteht. Zudem bietet eine solche Informationsveranstaltung eine Möglichkeit, diese Altersgruppe beim Weg in den Beruf zu begleiten und somit an die Fachgruppe und damit die VAAM zu binden.

Im Anschluss trafen sich 15 Teilnehmer abends in einem Tübinger Braukeller, um in gemütlicher Atmosphäre untereinander und mit dem Tübinger Regierungsinspektor für Arzneimittelüberwachung, Dr. Daniel Müller, die vielfältigen Arbeitsfelder als Mikrobiologe zu diskutieren (Biospektrum 4/12).

Im September trafen sich rund 30 aktive Mitglieder der Fachgruppe in Köln, um an einem attraktiven wissenschaftlichen Vortragsprogramm teilzunehmen. Die Veranstaltung fand in Anschluss an die „Aseptikon"-Konferenz statt, wodurch sich interessante Schnittmengen ergaben. Wir informierten uns zu Normentwürfen der Reinraumtechnik und der Nährmedienherstellung, erfuhren über Anwendungsfelder der Durchflusszytometrie, aktuelle Projekte und Produkte der Keimidentifizierung und Pyrogennachweise und diskutierten über Erwartungen seitens der Behörden zu „kritischen Keimen" in Pharmaprodukten. Zudem wurden Probleme und Lösungen bei der Keimidentifizierung seitens der Überwachungsbehörden anhand drastischer Fälle präsentiert. (Biospektrum 7/12)

Im Jahr 2013 treffen sich die Mitglieder der Fachgruppe zunächst in Bremen auf der Frühjahrstagung der VAAM. Hier werden wir am Montag, den 11. März 2013 nachmittags die Firma Bruker Daltronik GmbH besuchen, die Geräte zur MALDI-TOF-Analytik von Bakterien herstellt. Vortragende aus den Niederlanden und Deutschland werden die vielfältigen Problemstellungen der Keimanalytik mittels MALDI-TOF darstellen. Im Herbst findet unser Jahrestreffen an der Fachhochschule in Villingen-Schwenningen statt. Wir freuen uns bereits auf Ihre Vorträge, die Sie uns gerne jederzeit melden können. ■

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Fachgruppe Mikrobielle Zellbiologie

■ Das Symposium der Fachgruppe auf der Jahrestagung in Bremen ist das erste, nachdem unsere Mitgliederversammlung beschlossen hat, den zukünftigen Fokus auf zellbiologische Themen zu legen und den Namen der Fachgruppe entsprechend zu ändern. Unser Symposium widmet sich diesmal einem überaus klassischen Aspekt der Zellbiologie, der Kompartimentierung. Da die Frage nach der zellulären Organisation alle Prokaryoten betrifft, haben die Fachgruppen Archaea und Mikrobielle Zellbiologie vereinbart, die Veranstaltung dieses Jahr gemeinsam auszurichten.

Gibt es Kompartimentierung in Mikroorganismen? Die Autoren moderner Cytologie-Lehrbücher stellen gemeinhin fest, dass Prokaryoten nur aus einem einzigen (membranbegrenzten) Kompartiment bestehen. Sie leisten damit der Interpretation Vorschub, Mikroorganismen seien nicht oder kaum zellulär strukturiert. Diese Annahme geht gleich in zweierlei Hinsicht fehl. Denn Prokaryoten lassen sich nicht auf einen morphologisch einfachen Typ reduzieren, der als repräsentativ gelten und generell mit Eukaryotenzellen verglichen werden kann. So stellen Mikroorganismen mit einem intrazellulär ausgeprägten Membransystem zwar Spezialisierungen dar, sind aber keine Ausnahme. Zu diesem Thema haben wir drei Experten eingeladen, die über aktuelle Ergebnisse der Kompartimentbildung in Planctomyceten und Verwandten sowie in Archaeen berichten werden. Die aus der Historie der Zellbiologie herrührende Vorstellung greift zu kurz, Kompartimente seien immer von Lipidmembranen umgebene Räume, um abgegrenzte Zellregionen zu schaffen. Folgt man einem funktionellen Verständnis von Kompartimentierung, so findet man in Pro- und Eukaryoten Reaktionsräume unterschiedlichen Komplexitätsgrads von der makromolekularen bis zur zellulären Ebene. Der vierte Beitrag unseres Symposiums gibt einen Überblick über entsprechende Mechanismen und Strukturen und befasst sich mit dem Konzept der Kompartimentierung in Mikroorganismen.

Alle Mitglieder und Interessenten unserer Fachgruppe sind herzlich eingeladen, an der Mitgliederversammlung teilzunehmen und über die zukünftige Ausrichtung von Symposien zu beraten. Zeitpunkt und Raum finden Sie im Tagungsprogramm. Wir freuen uns auf Ihr Kommen! ■

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Fachgruppe Regulation und Signaltransduktion in Prokaryoten

Das Überleben in der mikrobiellen Welt verlangt von jeder Zelle ein kontinuierliches Überwachen zahlreicher extra- wie auch intrazellulärer Parameter, um rechtzeitig und adäquat auf Veränderungen reagieren und die zelluläre Physiologie entsprechend anpassen zu können. Zur Umsetzung solcher Prozesse benötigen Bakterien sensitive Sensoren und spezifische Regulatoren, die einen äußeren Reiz in eine zelluläre Antwort übersetzen. Zur Reizerkennung und -weiterleitung bedienen sich Prokaryoten hierbei Ein- und Zweikomponentensystemen, sowie alternativer Sigmafaktoren oder kleiner regulatorischer RNA-Moleküle. Daneben werden Signalstoffe aber auch aktiv von Bakterien produziert um zum Beispiel multizelluläre Differenzierungsprogramme sowohl extrazellulär über zelldichteabhängige Prozesse (*Quorum Sensing*), als auch intrazellulär mittels sekundärer Botenstoffe wie dem zyklischen di-GMP zu koordinieren.

Mit all diesen Facetten beschäftigt sich die Fachgruppe „Regulation und Signaltransduktion in Prokaryoten". Sie bietet allen an solchen Prozessen interessierten WissenschaftlerInnen innerhalb der VAAM ein entsprechendes Forum zum Erfahrungsaustausch und zum Vorantreiben von Kooperationsprojekten. Zu diesem Zweck organisiert die Fachgruppe jährliche Mini-Symposien während der VAAM-Tagung, die sich in den letzten Jahren mit verschiedenen Aspekten der Regulation und Signaltransduktion auseinandergesetzt haben. In diesem Jahr widmet sich das Mini-Symposium der Rolle von Proteinmodifikationen für die Regulation und Signaltransduktion. Hierfür konnten mit Haike Antelmann (Greifswald), Ivan Mijakovic (Paris), Kürsad Turgai (Hannover) und Alan Wolfe (Loyola, USA) vier hochkarätige Sprecher aus dem In- und Ausland gewonnen werden.

Eine weitere wichtige Aktivität der Fachgruppe ist die alle zwei Jahre stattfindende VAAM-Sommerschule, welche sich aus dem traditionsreichen Plasmidmeeting heraus entwickelt hat. Dieses Symposium hat sich in den letzten Jahren immer stärker zu einem Forum vor allem für NachwuchswissenschaftlerInnen entwickelt, das es ihnen ermöglichen soll, in einer anregenden und informellen Atmosphäre über ihre Forschung zu berichten und sich mit anderen zu vernetzen. Im Oktober vergangenen Jahres fand das Symposium „Mechanisms of Gene Regulation" zum 29. Mal statt und wurde von Mitarbeitern meiner Arbeitsgruppe und mir in Wartaweil am Ammersee ausgerichtet (BIOspektrum 6/2012).

Die Fachgruppe hat es sich auch zur Aufgabe gemacht, neue Forschungsrichtungen innerhalb ihres Feldes aufzuzeigen, sowie die Initiation neuer Forscherverbünde und Förderungsinitiativen zu unterstützen, wie zum Beispiel dem im vergangenen Jahr angelaufenen und von Kirsten Jung (München) koordinierten DFG-Schwerpunktprogramm „*Phenotypic heterogeneity and sociobiology of bacterial populations*".

Auch in diesem Jahr findet während der VAAM-Tagung in Bremen im direkten Anschluss an das Mini-Symposium eine Mitgliederversammlung der Fachgruppe statt, zu der ich Sie auf diesem Wege recht herzlich einladen möchte.

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Fachgruppe Umweltmikrobiologie

Die Fachgruppe Umweltmikrobiologie bietet ein breites Diskussionsforum von der mikrobiellen Ökologie über Prozesse und Metabolismus in der Umwelt, bis zur Umweltbiotechnologie. Nachdem die mikrobielle Ökologie enorme methodische Fortschritte gemacht hat, wurden in den letzten Jahren einige fundamental neue, ökologische Prozesse entdeckt, die das Leben auf der Erde nachhaltig beeinflussen. Die Fachgruppe möchte solche Themen aufgreifen und diskutieren und damit die mikrobiologische Umweltforschung in Deutschland stärken.

Wie jedes Jahr hatte die Fachgruppe Umweltmikrobiologie auf der VAAM Frühjahrstagung 2012 ein Mini-Symposium zu einem speziellen Thema organisiert. Die Session wurde von Barbara Morasch, Uni Tübingen geleitet mit der Frage: „Was macht organische Substanzen schwer abbaubar durch Mikroorganismen?". Eingeladene Redner waren Frederick Hammes, EAWAG und Fritz Widdel, MPI Bremen, der leider wegen Erkrankung nicht kommen konnte und durch einen Vortrag des Fachgruppensprechers Rainer Meckenstock ersetzt wurde. Weiterhin gab es einige exzellente Kurzvorträge.

Für die Tagung in Bremen 2013 organisieren wir eine gemeinsame Session mit der Niederländischen Fachgruppe „Microbial Ecology". Thema ist diesmal *„Elektrontransfer in the Subsurface"* und wir konnten mehrere exzellente Redner dafür gewinnen. Unter anderem wird es Vorträge zum neu entdeckten Prozess des Long-Distance-Electron-Transfer, Syntrophie und anderen Elektronentransferprozessen geben. Auch dieses Jahr freuen wir uns auf tolle Beiträge und lebhafte Diskussionen zu später Stunde.

Um der Fachgruppe noch mehr Leben einzuhauchen und die Umweltmikrobiologie in der VAAM zu stärken, wäre ein zusätzliches Symposium für das Jahr 2013 oder auch später willkommen. Wenn Sie Interesse haben, mit Unterstützung der Fachgruppe eine kleine Tagung/Workshop zu einem interessanten Thema zu organisieren, wenden Sie sich bitte an den Fachgruppensprecher.

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Fachgruppe Mikrobielle Pathogenität

Die Fachgruppe Mikrobielle Pathogenität schaut auf ein ereignisreiches Jahr 2012 zurück. Bis Ende 2011 umfasste sie über 300 Mitglieder und bildet damit inzwischen die zweitstärkste Fachgruppe der VAAM hinter der Umweltmikrobiologie. Zu Jahresbeginn wurden die VAAM-Sprecher der Fachgruppe Andreas Peschel (Tübingen) und Petra Dersch (Braunschweig) in einer per Email durchgeführten Wahl im Amt bestätigt. Die Jahrestagungen der VAAM in Tübingen und der DGHM in Hamburg hatten infektionsrelevante Schwerpunktthemen und wurden durch Mitglieder der Fachgruppe maßgeblich mitgestaltet. Im Juni wurde das traditionell alle zwei Jahre stattfindende Fachgruppensymposium in Bad Urach mit großer Resonanz durchgeführt. In diesem Rahmen wurde auch der von Sanofi gespendete Preis der Fachgruppe an Patrick Kaiser (Frankfurt) verliehen. Die Fachgruppe unterstützte zudem kleinere Symposien zu spezifischen Themen der Mikrobiellen Pathogenität wie das von Forschungsverbünden aus Bonn (FOR854), Würzburg (SFB630) und Tübingen (SFB766) gemeinsam organisierte Doktorandensymposium „New drugs and targets to fight against infectious diseases“ im November in Freudenstadt. Auch 2013 wird die Fachgruppe aktiv die Zusammenarbeit zwischen VAAM und DGHM fördern und Tagungen und Workshops mit organisieren oder fördern. Neben den beiden Jahrestagungen in Bremen (VAAM) und Rostock (DGHM) werden mit Unterstützung der Fachgruppe der 11. Deutsche Chlamydienworkshop im April in Würzburg, das zweite Symposium des Tübinger SFB766 „The bacterial cell envelope: structure, function, and infection interface“ vom 6. bis 8. Mai in Irsee und die dritte internationale Konferenz „Regulating with RNA in bacteria“ vom 4. bis 8. Juni in Würzburg stattfinden.

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VAAM-DECHEMA-Fachgruppe Biotransformationen

Die 1996 gegründete VAAM Fachgruppe „Biotransformationen“ mit derzeit rund 150 Mitgliedern ist seit November 2008 eine gemeinsame Fachgruppe der VAAM und der DECHEMA (http://biotech.dechema.de/Biotrafo.html). Innerhalb der DECHEMA gehört sie neben den gemeinsamen Fachgruppen Bioprozesstechnik (DECHEMA-GVC) und Chemische Biologie (DPhG, GBM und GDCh) zusammen mit zehn weiteren Fachgruppen zur DECHEMA Fachgemeinschaft Biotechnologie. Der Fachgruppenbeirat (Vorsitzende Christoph Syldatk, Karlsruhe, und Andreas Liese, Hamburg-Harburg) umfasst als Lenkungsgremium derzeit 34 Mitglieder. Im Jahr 2014 wird dieser (dann 20 köpfige) FG-Beirat von allen FG-MitgliederInnen neu gewählt werden, nähere Infos werden noch bekannt gegeben. In der gemeinsamen FG sind neben Mitgliedern aus der Industrie maßgeblich auch Vertreter der Gesellschaft Deutscher Chemiker (GDCh) und der Gesellschaft für Fettwissenschaften (DGF) engagiert. Dieses Zusammengehen verschiedener Fachdisziplinen lag nahe: Die wachsende Nachfrage nach ökonomischen, ökoeffizienten und ressourcenschonenden Prozessen in der Chemie-, Pharma-, Energie- und Lebensmittelindustrie erfordert verstärkte Anstrengungen, Forschungsergebnisse in Produkte umzusetzen. An biokatalytischen Verfahren führt dabei kein Weg vorbei, sie bilden die Grundlage der modernen Industriellen Biotechnologie, und ihre biologische Basis ist die anwendungsorientierte Mikrobiologie. Durch die gemeinsame VAAM-DECHEMA FG „Biotransformationen“ kann dieses Arbeitsgebiet wesentlich besser und effektiver vertreten werden. Es können nun Forscher aus Universitäten, Forschungseinrichtungen, der chemischen und der pharmazeutischen Industrie noch enger zusammengeführt werden, was bereits in einer Reihe von Veranstaltungen gemeinsam mit anderen Gremien der DECHEMA und Fachgruppen der VAAM geschehen ist. Ziel dabei ist vor allem, durch spezielle Symposien für Doktoranden, Habilitanden und Juniorprofessoren junge Forscher zu fördern. Ein Highlight der Aktivitäten 2012 war die Organisation der Tagung *„Catalyzing Bio-Economy: Biocatalysts for Industrial Biotechnology“* (24./25. April 2012, Frankfurt/M.). Beteiligt war die FG auch an der Organisation des DECHEMA Frühjahrestreffens der Biotechnologen (4./5. März 2013, Frankfurt/M.). Für die VAAM Frühjahrestagung in Bremen freut es uns sehr, zum Symposium „Microbes and Catalytic Biofilms: Industrial Catalysts of the Future?“ am Montagabend und am Dienstagmorgen einladen zu können.

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Microbiology at the universities and research institutes in the Federal State of Bremen

With more than 200 scientists in the field of microbiology, the Federal State of Bremen has developed its scientific endeavors quite strongly in the past years. Microbiology is an academic research topic at the University of Bremen, the Jacobs University, the University of Applied Sciences, the Hochschule Bremerhaven, the Leibniz Center for Tropical Marine Ecology, the MPA Bremen Institute for Materials Testing, the Max Planck Institute for Marine Microbiology, the Alfred Wegener Institute for Polar and Marine Research and in several industries. Many of these institutions pool their teaching and research activities in the joint program of the International Max Planck Research School MarMic. This training school is attracting many students from all over the world.

University of Bremen

www.uni-bremen.de

The University of Bremen is a campus university with about 20000 students and a broad array of disciplines. Its research profile is shaped to a great extent by marine and climate research in the natural sciences, engineers in the field of production and manufacturing technology, and the social sciences. These main areas encompass four collaborative research centers, the DFG research center 'Ocean Margins', a cluster of excellence, two graduate schools of excellence and several research training groups. The designation of six high-profile areas further enhances the University's profile, which is rounded off by cooperation with the non-university institutes belonging to research societies like Max-Planck, Helmholtz, Fraunhofer and Leibniz. The close proximity of many non-university research institutions opens up possibilities for intensive cooperation on research projects and there are currently around 30 joint professors. In June 2012 the University of Bremen received the status "Exzellenz-Universität".

Faculty of Biology/Chemistry
Microbial Ecophysiology
Prof. Dr. Michael W. Friedrich and Prof. Dr. Karl-Heinz Blotevogel

The Microbial Ecophysiology group of Michael Friedrich at the Faculty of Biology and Chemistry, University of Bremen, focuses on linking structure ("who is there") to function ("what are they doing") of microbial communities. The group investigates microorganisms that respire anaerobically (e.g., dissimilatory iron reducers, methanogens, dehalogenating microorganisms), play an important role in the carbon flow through microbial communities in sediments and soils, or use an anode in microbial fuel cells as electron acceptor. Central to their approach is the state-of the-art community level analyses with molecular tools (e.g., stable isotope probing of nucleic acids) in combination with an assessment of the underlying biogeochemical processes, and environmental parameters.

Marine Microbiology
Prof. Dr. Ulrich Fischer

Ulrich Fischer's group Marine Microbiology focused on the sulfur metabolism and physiology of marine oxygenic and anoxygenic photothrophic bacteria, as well as bioactive substances of marine heterotrophic bacteria. The research group ended when he retired in 2010. Nevertheless, Ulrich Fischer is still active as a consultant and with his expertise he continues acting as a reviewer for the scientific community.

Institute of Ecology, Botany
Prof. Dr. Uwe Nehls

Uwe Nehls is head of the Botany at the University of Bremen. His group studies ectomycorrhizas, symbiotic associations of soil fungi and roots of many forest trees. The research topic covers functional genomics of ectomycorrhizal model fungi and the tree model poplar. Main aspects are cellular organization of carbohydrate and nutrient exchange and deciphering the molecular cross-talk between fungus and plant. Among the methods applied are transcriptome, proteome and metabolome analyses of plant and fungal partners. Additionally, studies of transport proteins by heterologous expression and visualization of gene expression and protein function in plant and fungi are of major interest.

Department of Microbe-Plant Interaction
Prof. Dr. Barbara Reinhold-Hurek

Barbara Reinhold-Hurek is head of the Department of Microbe-Plant Interaction at the University of Bremen. Her group studies the model system endophytic diazotrophic bacteria (*Azoarcus spp.* and related genera) and *Gramineae* such as rice and Kallar grass, particularly the molecular mechanisms of plant-bacteria interactions. The research topics cover functional genomics of bacteria and rice, the detection and analysis of bacterial gene expression, deciphering the molecular cross-talk, colonization and host recognition as well as signal transduction cascades of both partners. Among the methods applied are transcriptome, proteome and mutational analyses. Additionally, the functional diversity of endophytic diazotrophs, community structure

and activity in situ by cultivation-independent molecular techniques, classical isolation techniques, and polyphasic taxonomy are of interest. Furthermore, the research concentrates on the regulation of bacterial gene expression (transcriptome and proteome analysis, genetic and biochemical analysis of signal transduction cascades).

Department of Virology
Prof. Dr. Angelika Vallbracht and
Prof. Dr. Andreas Dotzauer

The research focus of the Department is on the pathogenesis of viral infections, with the emphasis on biochemical, molecular biological and immunological studies of the virology of the hepatitis A virus (HAV). The main interests are the mechanisms responsible for the hepatotropism of HAV (IgA-carrier hypothesis) as well as the interactions of HAV with the host cells during entry, replication and release and the interference of HAV with the innate immune response and the hematopoietic system during viral infection. The research of the Department is generously supported by the Tönjes-Vagt-Foundation, which funds basic virus research in Bremen.

MARUM-Center for Marine Environmental Sciences at the University of Bremen

www.marum.de

Organic Geochemistry Group
Prof. Dr. Kai-Uwe Hinrichs

Hinrichs' group studies the interactions between microbial life and the carbon cycle on a range of spatial, temporal and molecular scales. The group examines which and how microbes shape marine element cycles and what the related environmental consequences are. In order identify and ideally quantify microbial processes, the researcher decipher the information encoded in distributions and isotopic compositions of organic biomarker molecules in geological and environmental samples. The group consists of closely collaborating biologists, chemists, geochemists, and marine geoscientists. In research projects, analyses of environmental samples are combined with experimental, laboratory-based approaches. The current research foci encompass the deep subsurface biosphere, methane biogeochemistry, life in extreme environments, development and application of new geochemical-analytical techniques, prokaryotic membrane lipid taxonomy, and the study of paleoenvironments associated with major perturbations of the C-cycle. The funding sources include an ERC Advanced Grant and a Gottfried-Wilhelm Leibniz Price (DFG).

Emmy-Noether Young Investigator
Research Group Hydrothermal Geomicrobiology
Dr. Solveig Bühring

The group of Bühring focuses on the exploration of organisms inhabiting submarine shallow-water hydrothermal vent systems. Their study is of great significance to geochemistry, geobiology and microbiology, because they act as windows into the Earth and its multiple linkages between the bio- and geosphere. The limits of life as well as its potential origin are two of the greatest puzzles in biogeosciences, which one can address via the study of hydrothermal systems. The central research strategy is based on the information encoded in structural and isotopic properties of membrane lipids of the indigenous organisms, in combination with stable isotope probing experiments investigating uptake of tracer into biomarker and rRNA.

Max Planck Institute for Marine Microbiology

www.mpi-bremen.de

Max Planck scientists analyze how marine microbes convert substances and what these processes signify for the global element cycles of carbon, sulfur, nitrogen and iron. They concentrate on reactions that have already been discovered, including the anaerobic oxidation of methane (AOM), anaerobic ammonium oxidation (anammox) and the reduction of sulfate. Also they look for new, hitherto unknown metabolic pathways. Their efforts are also aimed at discovering how prokaryotes have adapted to their environment and respond to changes, and the effects of all this on their habitat, the earth and the climate. On the field of teaching and research an intense collaboration exists between the Max Planck researchers and the Bremen Universities.

Department of Biogeochemistry
Prof. Dr. Marcel Kuypers

Fundamental research in the Department of Biogeochemistry headed by Marcel Kuy-

Die Universität aus der Luftperspektive © Universität Bremen

pers addresses the microbial cycling of biolimiting elements in the ocean, which controls to a large extent the chemistry of the earth's ocean and atmosphere. Understanding the pathways, interactions and environmental regulation of microbial processes that control the availability of biolimiting nutrients such as nitrogen, phosphorus and iron, in the ocean is imperative to predict the impact that human activities will have on the chemistry of our ocean and climate.

Microsensor Group
Dr. Dirk de Beer

The Microsensor Group headed by Dirk de Beer studies the functioning of microbial communities in sediments, microbial mats and biofilms with high spatial resolution techniques. With needle shaped microsensors and planar optodes dynamics of substrates are monitored with a spatial resolution of microns and a time resolution of seconds. The microsensors have a tip size of ca 5 micron, thus the measurements reflect the undisturbed situation. Main topics are the regulation of oxygen, nitrogen, sulfur and carbon cycles by photosynthesis and mineralization processes.

Department of Microbiology
Prof. Dr. Friedrich Widdel and
Prof. Dr. Jens Harder

The group of Friedrich Widdel studies the degradation of long-lived natural organic compounds as well as aquatic microbial redox reactions of inorganic sulfur, nitrogen and iron species. Jens Harder's Diversity Group explores the biogeography of marine planctomycetes, genus *Rhodopirellula*, as well as growth of previously uncultivated microorganisms from pelagic and benthic habitats, often in collaboration with the Molecular Ecology group. Furthermore, the biochemistry of anaerobic monoterpene utilization is of interest.

Department of Molecular Ecology
Prof. Dr. Rudolf Amann,
Prof. Dr. Nicole Dubilier and
Prof. Dr. Frank Oliver Glöckner

The Department of Molecular Ecology, headed by Rudolf Amann, directs its work towards understanding the structure and function of marine microbial communities. There is a special interest in the dynamics of phylogenetically defined bacterial clades with regard to biotic and abiotic changes in the planktonic or benthic environment. Much of the work is focused on nucleic acids. Molecular methods like fluorescence in situ hybridization or metagenomics are first optimized in more defined systems like biofilms or symbiotic associations, and finally adapted to the study of highly complex microbial systems.

The Symbiosis Group of Nicole Dubilier studies the biology and ecology of associations between bacteria and eukaryotes, with the main emphasis on marine invertebrates from chemosynthetic environments such as sulfide-rich coastal sediments, hydrothermal vents and cold seeps.

The Microbial Genomics and Bioinformatics Research Group of Frank Oliver Glöckner develops enabling technologies to transform the wealth of sequence- and metadata from the environment into biological knowledge. Techniques are whole genome and metagenome analysis, sequence classification, phylogenetic inference as well as software and database development for integrated data analysis. An integrated view on the complex interplay of organisms, genes and the environment surrounding them is the first step towards the statistical analysis and modelling of complex metabolic processes and networks (ecosystems biology).

Max Planck Research Groups

Marine Geochemistry
Dr. Thorsten Dittmar

The Max Planck Research Group for Marine Geochemistry headed by Thorsten Dittmar was established at the Institute for Chemistry and Biology of the Marine Environment (ICBM, Carl von Ossietzky University, Oldenburg) in September 2008 in a collaborative effort with the Max Planck Institute for Marine Microbiology. The instrumental highlight of the group is an ultrahigh-resolution mass spectrometer (15 Tesla Fourier-Transform ion cyclotron resonance mass spectrometer, FT-ICR-MS) making it a unique analytical facility in the marine sciences. Main research focus of the group is dissolved organic matter (DOM) in the ocean. The ocean is one of the largest carbon reservoirs on Earth. Dissolved organic matter alone contains a similar amount of carbon as all living biomass in the ocean and on land combined. Even though marine DOM is mainly of microbial origin, its turnover in the ocean is remarkably slow. Dissolved organic matter has accumulated in the ocean over thousands of years, and the controlling mechanisms behind its turnover and cycling are largely unknown. Advanced molecular techniques, in particular ultrahigh-resolution mass spectrometry, are used in the research group to obtain answers to the fundamental questions regarding the cycling of organic matter in the oceans.

Microbial Fitness Group
Prof. Dr. Marc Strous

The Microbial Fitness Group headed by Marc Strous addresses the molecular basis of the

ecological fitness of bacteria. The aim is to understand how the environment selects for specific microbial processes, organisms and genes. They approach this problem from a System Sciences perspective and proceed from first principles: Chemistry and Thermodynamics. The experimental approach consists of the sampling of natural microbial communities, the incubation of these communities in laboratory bioreactors and the monitoring of community dynamics by -omics and in situ approaches. This is a unique combination, that enables both complete control over environmental conditions and a comprehensive characterization of the resulting microbial communities. Results are interpreted in the context of a mathematical model of communal metabolism. The microbial nitrogen cycle is currently the main focus. This research is a joint effort of the Max Planck Institute for Marine Microbiology and the Center for Biotechnolgy (CeBiTec), University of Bielefeld. The group is supported by an ERC starting grant.

International Max Planck Research School of Marine Microbiology

www. marmic.mpg.de

Coordinator Dr. Christiane Glöckner

The International Max Planck Research School of Marine Microbiology (MarMic) was established in 2002 as a joint MSc/PhD program of the Max Planck Institute for Marine Microbiology with the University of Bremen, the Jacobs University Bremen, and the Alfred-Wegener-Institute for Polar and Marine Research. MarMic is one of the few graduate schools worldwide that is able to offer a graduate degree in marine microbiology because of the singular concentration of expertise in disciplines relevant to marine microbiology in and around Bremen. The MarMic program consists of intensive theoretical and practical training in biogeochemistry, marine chemistry and physics, prokaryotic and eukaryotic microbiology, molecular ecology, microbial physiology and metabolism, genetics and bioinformatics, and is thus truly interdisciplinary, bridging life, environmental, and geological sciences. Rudolf Amann acts as the spokesperson, and the coordinator is Christiane Glöckner. Further support comes from Karl-Heinz Blotevogel (University Bremen Coordinator of the MSc program) and from Jens Harder. All groups within the MPI Bremen and the MarMic institutions contribute to the school with teaching. The active faculty staff is now at 42 professors and lecturers

Jacobs University

www.jacobs-university.de

Jacobs University is a private residential university offering education leading to various Bachelor, Master, and PhD degrees. The campus university hosts an international student body coming from more than 110 different nations. Interdisciplinary education and research is organized in clusters of faculty from natural sciences, engineering, and the humanities. Excellence and transdisciplinarity, diversity and community, as well as leadership values are the pillars of a Jacobs education.

Molecular Biotechnology

Prof. Dr. Roland Benz

The Working Group Molecular Biotechnology headed by Roland Benz is interested in two different topics. One topic focuses on the interaction of membrane-active molecules with biological and artificial membranes. This research is based on the observation that small hydrophilic molecules and ions have very low solubility in organic phases due to the considerable energy needed for their transfer from the aqueous phase into the low dielectric interior of biological and artificial membranes formed by the hydrocarbon side chains of lipids. As a consequence carriers and channels are needed for controlled transport of hydrophilic solutes through membranes. We are interested in the biophysics of membrane transport across the cell wall of gram-negative and gram-positive bacteria. For this cell wall channels are isolated from these bacteria. Their properties are characterized in reconstituted membrane systems. To study the properties of the cell wall channels in detail we perform also site-directed mutagenesis of these channels. Of special interest in recent years was the study of cell wall channels from the mycolata in artificial membranes. The second topic of the Benz group is the work on different projects in environmental biotechnology. One project is the study of biogas production by anaerobic fermentation of biomass. Of special interest is the control of biogas production by on-line monitoring of volatile fatty acids in the bioreactor and the use of alternative substrates such as marine biomass that are not in competition with the production of food for humans and animals. The other project in environmental biotechnology is the further development of a control system for waste-water cleaning plants, which allows optimal removal of nitrogen from the waste-water at very low energy consumption. The control system is based on the fuzzy-logic computer language. Input for control and regulation are provided by only two measures: the oxygen concentration and the redox potential.

Molecular Genetics

Prof. Dr. Georgi Muskhelishvili

The Working Group Molecular Genetics headed by Georgi Muskhelishvili is interested in genetic regulation. Genetic regulation can be considered a genuine device optimising the cellular adaptation by coordinating the chromosomal gene expression in space and time. Uncovering the mechanisms coordinating the spatial-temporal gene expression is therefore central for understanding genetic regulation. The relatively small size of the chromosome and the limited number of genes make the bacterial cell an excellent model system to investigate the general organizational principles of the genetic regulation and especially, its flexibility in sustaining the phenotypic responses to the changing environment. The research of the group, traditionally carried out in the classical model organism *Escherichia coli*, has been recently extended to plant (*Dickeya dadantii*) and human (*Pseudomonas aeruginosa*) pathogens. The aim is to understand how the systemic-organisational pro-

Die Schlachte, Zeichnung von Manfred Schlösser, www.manfredschloesser.de

perties of the genetic system are reflected in the relationships between the structure and function of the chromosome during both, normal and pathogenic growth. For this purpose the group developed a novel holistic methodology combining the methods of molecular genetics, biochemistry and cell biology (Atomic Force Microscopy and Laser Scanning Microscopy) with transcriptomics, proteomics, metabolomics and bioinformatics approaches. This integrative approach enables to describe the chromosomes as thermodynamic machines converting available metabolic energy into information.

Molecular Microbiology
Prof. Dr. Matthias Ullrich
The Molecular Microbiology group of Matthias Ullrich is studying molecular and cellular interactions of heterotrophic bacteria with photosynthetic eukaryotic hosts in context of environmental adaptations. Those host organisms are terrestrial crop plants, mangroves, and marine diatoms. The major prokaryotic model organisms are *Pseudomonas syringae*, *Marinobacter adhaerens*, *Erwinia amylovora*, and the nitrogen-fixing *Marinobacterium* sp. These microbes are genetically accessible and subject to molecular genetics experiments such as gene-specific mutagenesis, proteomics, enzyme analysis, and reporter gene expression analysis. While the plant-associated projects mainly deal with bacterial virulence and fitness, the marine topics concentrate on mechanisms of aggregate formation. In the focus of our model organism-based research are topics such as bacterial gene expression, exopolymer synthesis, protein secretion, quorum sensing, multidrug efflux systems, cell-to-cell adherence, biofilm formation, nitrogen fixation, and the analysis of plant-borne bio-active compounds.

Environmental Microbiology
Dr. Helge Weingart
The goal of the research of Helge Weingart is to identify and characterize multidrug efflux (MDE) pumps in the plant pathogenic bacteria *Pseudomonas syringae* and *Erwinia amylovora* and to gain in-depth knowledge about their regulation and natural functions. These bacteria infect different plants, each of which produce a unique spectrum of antimicrobial metabolites. MDE pumps may play an important role in the adaptation of plant pathogenic bacteria to their respective host plants by protecting them against plant antimicrobials. The multiplicity of multidrug transporter in the bacterial genomes is probably due to the fact that they are fulfilling a broad diversity of roles in the export of different hydrophobic substrates and many of them may only transport drugs opportunistically because of the accommodating nature of their substrate binding pockets. To learn more about their natural functions and their role during pathogenesis, we analyze the expression of these transporters during infection of the host plant. Moreover, the genes encoding such pumps can easily be interchanged between bacterial species leading to new multi-resistant pathogens. Multi-resistant pathogens are profoundly important to human health, but the environmental reservoirs of resistance determinants are poorly understood.

Bremen University of Applied Sciences

www.hs-bremen.de
The Bremen University of Applied Sciences enjoys a long tradition that goes back to 1799. The range of degree courses is decidedly innovative and practically oriented.

Approximately 8 000 students enroll in 70 degree courses in areas such as engineering, natural sciences, economics and social sciences.

Institute of Environmental Engineering, Ecology and Biotechnology
Prof. Dr. Bernd Mahro
The research of Bernd Mahro (Environmental Microbiology) focussed for a long time on environmental biotechnology aspects especially in the field of soil and groundwater remediation, elucidating biological and environmental parameters that influence the microbial degradation of xenobiotics in soil or groundwater. The actual work deals primarily with bioconversion strategies for biogenic residues from the food industry (valuables from waste).

Industrial Microbiology
Prof. Dr. Tilman Achstetter
Prof. Dr. Tilman Achstetter is interested in aspects of genetic stability in *Saccharomyces cerevisiae*-based recombinant model systems. The work deals with the setup of a sensitive *in vivo*-detection method of plasmid loss. Multicopy plasmids, used frequently in industrial production involving yeast, carry a segment of the endogenous 2 μm plasmid and rely on functions of this plasmid in terms replication and partitioning. Potential recombination events between the 2μm and hybrid plasmids are being traced.

Institute for Materials Testing (MPA) Bremen

Dr. Jan Küver
www.mpa-bremen.de
The Institute for Materials Testing (MPA) Bremen provides services of work on the field of metal and building material testing and damage analysis. Microorganisms, such as bacteria, fungi and algae, are ubiquitous in our environment. They colonize materials of all kinds causing changes of material properties, degradation or even destruction of the materials. The Department of Microbiology headed by Jan Küver is involved in projects of applied research dealing with studies of basic microbiological processes on the colonization and degradation of materials including technical liquids (e.g. MIC, cultural heritage, coatings and paintings, metal working fluids), analyses of microbially induced damages of materials and the development of concepts to reduce or prevent these damages.

Bremerhaven University of Applied Sciences

www.hs-bremerhaven.de/
Bremerhaven University of Applied Sciences can look back on over 100 years of education tradition. Today, the University has about 2.600 students. The curricula and research activities of the courses are targeted at what is needed in practice and gives this new impetus through future-oriented developments. Most diploma theses are compiled in close cooperation with regional businesses and contribute to strengthening the competitiveness of the economy. This mutual exchange of ideas however also has a stimulating effect on teaching

Prof. Dr. Matthias Nagel
The applied research is focused on the application of lactic acid bacteria for stabilization of liquid feed and by-products to reduce spoilage and the use of antibiotics in feeding of animals. Additional projects involve screening, isolation, and characterization of bacterial enzymes for application in animal feed and the development and improvement of microbiological methods for detection of special spoilage organisms in food and feed.

The Alfred Wegener Institute for Polar and Marine Research (AWI)

www.awi.de

The Alfred Wegener Institute for Polar and Marine Research (AWI) was established in 1980 and is a member of the Helmholtz Association of German research centres. It is an internationally recognized centre for polar and marine research and makes significant contributions to Earth system and climate research in polar regions and coastal waters. Recent additional research themes include North Sea Research, contributions to Marine Biological Monitoring, Marine Pollution Research, Investigation of naturally occuring marine substances and technical marine developments. The Institute's research mission is to improve the understanding of ocean-ice-atmosphere interactions, biodiversity and function of all size classes of life, biogeochemistry and the evolution of the polar continents and seas. Global change research is a central focus. AWI collaborates in numerous international research programmes and maintains close contacts with many universities and institutes in Europe and farther afield. It operates the research ice breaker "Polarstern", as well as a number of polar and coastal field stations.

Die Bremer Stadtmusikanten, Zeichnung von Manfred Schlösser, www.manfredschloesser.de

HGF MPG Joint Research Group for Deep-Sea Ecology and Technology
Prof. Dr. Antje Boetius

The Helmholtz – Max Planck Joint Research Group for Deep-Sea Ecology and Technology headed by Antje Boetius has laboratories and offices at the AWI and at the MPI in Bremen, and studies polar and temperate deep-sea ecosystems. A main focus is the biogeochemistry and diversity of the Arctic deep-sea and its responses to the rapid sea ice retreat and ocean warming. The group operates a long term ecological time series site HAUSGARTEN in Fram Strait. Another focus are extreme microbial habitats in the ocean – from the ice-covered deep-sea basins of the Central Arctic to hydrothermal vents and cold seeps world wide. Actual research approaches encompass the measurement and modeling of diffusive and advective processes in diverse submarine habitats, the in situ quantification of transport and reaction, as well as the comparative analysis of life in extreme environments such as mud volcanoes, hot vents and gas hydrates. The goal of our research on "microbial habitats" is to understand niche formation and to investigate regulatory mechanisms for the occurrence and distribution of microbial populations. This requires the development of a variety of in situ techniques, as well as experimental strategies to quantify the nature and variability of the habitat on different temporal and spatial scales.

Ecological Chemistry
Prof. Dr. Allan Cembella and Dr. Elisabeth Helmke

The Ecological Chemistry Section headed by Allan Cembella sets its research goals to define and explain the functional role of natu-

rally occurring and anthropogenic chemical compounds and their biological interactions in marine ecosystems. The approach is to address the structural and functional complexity of chemical constituents not only by classical marine chemistry, but also from the functional perspective of biological-chemical interactions (chemical ecology). Chemical signals and effects are postulated to play a crucial role in maintaining the integrity, diversity and stability of marine communities. The scientists therefore concentrate on the structural-functional relationships of key primary metabolites (e.g. lipids), natural products (particularly marine bioactives), natural and anthropogenic toxins, and complex dissolved organic matter.

Two microbiologically oriented research groups have been established within the multidisciplinary Section Ecological Chemistry. The Polar Marine Microbiological group of Elisabeth Helmke focusses on bacterial communities in polar environments, particularly associated with sea ice, glacial ice, and deep sea habitats. Microbiological, ecological and molecular biological techniques are applied to understand the ecological roles, functioning, and adaptations to extreme conditions, as well as to describe the diversity, community structures and physiological capabilities of marine bacteria. These studies are carried out with natural environmental samples and bacterial isolates. Bacterial isolates collected from marine polar environments and the marine actinomycetes are also the basis for screening programs for new natural products and enzymes for diverse applications.

The mission of the research group on Chemical Ecology of Marine Protists and Cyanobacteria led by Allan Cembella is the characterisation of bioactive metabolites, their mode of action in species interactions, and the genetic regulation of biosynthesis and growth for determination of ecological function and role in population dynamics of marine plankton. The research group has three major research foci: molecular and evolutionary ecology (Uwe John), analytical and natural products chemistry of bioactive metabolites (Bernd Krock), and protistology and species interactions (Urban Tillmann). The research concentrates primarily on eukaryotic microalgae (protists), particularly toxic and/or allelochemically active planktonic species, while key toxigenic cyanobacteria serve as model systems for biosynthesis and gene expression studies. Chemically mediated mechanisms involved in determining population structure, diversity, growth, survival and co-evolution in marine food webs, including grazer interactions are the major research interest.

Helmholtz-University Young Investigators Group Planktosens
Dr. Katja Metfies

The Helmholtz Young Investigators Group PLANKTOSENS headed by Katja Metfies is a cooperation of the Alfred Wegener Institute for Polar- and Marine Research and the Jacobs University Bremen. The research of the group is dedicated to elucidate climate change consequences at the base of marine food webs in Polar Regions and the North Sea. This involves assessments of diversity, biogeography and succession patterns of eukaryotic phytoplankton, including the pico- and nanoplankton taking advantage of molecular methods. Furthermore, molecular sensing technologies are under development and integrated into a smart observation strategy to cut down costs and effort involved in the observation of marine phytoplankton.

Marine Microbial Ecology Group
Prof. Dr. Karen Wiltshire,
Dr. Gunnar Gerdts, Dr. Antje Wichels, and Dr. Ingeborg Bussmann

At the Biological Station Helgoland as part of the AWI the Marine Microbial Ecology Group is working on microbial diversity and activities in the marine environment with focus on marine costal areas. At present, they concentrate mainly on the North Sea, with its diverse habitats focussing especially on how different bacterial groups and populations are influenced by climate change. Accordingly they have recently expanded also into polar and brackish environments. The Marine Microbial Ecology group at Helgoland (Biologische Anstalt Helgoland) aims at an understanding of the microbial diversity on spatial and temporal scales and their changes in the marine coastal environment. We concentrate mainly on coastal areas of the North Sea, focusing on how different bacterial groups and populations are influenced by climate change and anthropogenic impacts. Current topics are the identification and biogeography of potentially pathogenic bacteria (*Vibrio* spp.). Another important issue in this context is long term ecological research (LTER) focusing on changes in the bacterial community structure on larger time scales. Since bacteria serve as food source for a number of organisms, our reseach is also linked to higher trophic levels (i.e. phytoplankton, zooplankton, and mussels). Foodweb interactions are currently studied with a focus on gelatinous plankton. Actually and as an evolving important field, the new topic microplastics is addressed starting with basic analysis of occurrence, abundances and sinks of the particles in coastal systems.

Leibniz Center for Tropical Marine Ecology

www.zmt.bremen.de

Tropical Marine Microbiology Group
Dr. Astrid Gärdes

The Tropical Marine Microbiology Group investigates the effects of eutrophication on microbial community response. We are especially interested in microbial interactions between heterothrophic bacteria and phytoplankton relevant in marine aggregate formation and particle sedimentation rates impacting tropical reef communities. One major source of eutrophication is extensive fish farming- aquaculture- in tropical coastal areas which leads to the disappearance of coral reefs, mangroves and sea grass meadows. Ultimately understanding the ecological impact of aquaculture on microbial associations can provide a scientific basis for tropical costal management.

Sunday, 10 March 2013

	Hanse-Saal
15:00–15:30	Welcome Addresses **p. 33**
15:30–16:30	**Public Lecture** *EHEC-Bakterien – gestern und heute* **p. 33**
	Coffee break/Industrial exhibition
17:00–18:00	**Plenary Session I** *Marine microbiology* **p. 33**
18:00–19:00	**Plenary Session II** *Unicellular eukaryotic microbiology* **p. 33**
ca. 19:00	**Welcome Reception**
	Industrial exhbition (Hall 4.1)

Monday, 11 March 2013

	Hanse-Saal	Kaisen-Saal	Borgward-Saal	Focke-Wulf-Saal	Lloyd-Saal	Salon Danzig	Salon Franzius	Salon London	Salon Bergen	Bruker Daltonik GmbH
08:30–10:30		Physiology I	ISME-Session (Marine Microbes I)	Archaea	Omics for biodiversity, biotechnology and bioinformatics research	Biochemistry: Structure, pathway, and compounds	Bacterial signal transduction: From individual genes to multicellular communities	Structural and functional dynamics of the gut microbiota in interaction with the host	Food, feed, fuel – Microbes in action	
		p. 42	p. 42	p. 42	p. 43	p. 43	p. 43	p. 44	p. 44	
	Coffee break/Industrial exhibition									
11:00–12:45	**VAAM Honorary Award & Plenary Session III** Host-microbe interaction									
	p. 33									
	Coffee break/Industrial exhibition									
14:15–15:15	**Plenary Session IV** Single cell microbiology									Maldi TOF and other modern identification techniques; lab visit and presentations
	p. 34									p. 36
15:15–17:30		**15:30–17:00 Karriere-Symposium**	Poster Session I (odd poster numbers)/Coffee break/Industrial exhibition							
		p. 11								
17:30–19:30		Electron transfer - processes in the subsurface	Protein modifications in gene regulation and signal transduction	Pathogen adaptation to host organisms	Microbial ecology of mice and man	Microbes and catalytic biofilms: Industrial catalysts of the future?	Microbial compart-mentalization	Challenges in microbial identification		
		p. 36	p. 37	p. 38	p. 39	p. 39	p. 40	p. 40		

Special Group Mini-Symposia Short Lecture Special Session

Tuesday, 12 March 2013

	Hanse-Saal	Kaisen-Saal	Borgward-Saal	Focke-Wulf-Saal	Lloyd-Saal	Salon Danzig	Salon Franzius	Salon London	Salon Bergen
08:30–10:30		Physiology II	Bacterial cell biology	Geomicro-biology: From rocks to communities and genomes I	Microbes and their environments	Microbes and catalytic bio-films: Industrial catalysts of the future?	Polar mirobial ecology/ Marine microbes II	Dissecting virulence I	Bacterial regulation and small non coding RNAs
		p. 45	p. 45	p. 46	p. 46	p. 41	p. 46	p. 47	p. 47
	Coffee break/Industrial exhibition								
11:00–12:00	**Plenary Session V** *Omics and bioinformatics*								
	p. 34								
12:00–13:00	**Plenary Session VI** *Environmental biotechnology*								
	p. 34								
13:00–14:30	Lunch break/Industrial exhibition		Industrial Lunch Symposium *Pacific Biosciences*	Lunch break/Industrial exhibition					
			p. 14						
14:30–15:30	**Plenary Session VII** *Physiology and metabolism*								
	p. 35								
15:30–17:30	Poster Session II (even poster numbers)/Coffee break/ Industrial exhibition			MIRRI	Poster Session II (even poster numbers)/Coffee break/Industrial exhibition				
				p. 48					
17:00–19:00		VAAM Annual General Meeting & PhD Awards							
ca. 19:30	**Mixer**								
	Weserstadion Bremen (see p. 8)								

Special Group Mini-Symposia | Short Lecture | Industrial Lunch Symposium | Special Session

Wednesday, 13 March 2013

	Hanse-Saal	Kaisen-Saal	Borgward-Saal	Focke-Wulf-Saal	Lloyd-Saal	Salon Danzig	Salon Franzius	Salon London
09:00–11:00		Biochemistry, Metabolism	Industrial biotechnology	Proteomics and metabolomics – From communities to cells to pathways	Molecular ecology and techniques	Plant pathogens/ Geomicrobiology	From soil to the ocean	Dissecting virulence II
		p. 48	p. 48	p. 49	p. 49	p. 50	p. 50	p. 50
	Coffee break/Industrial exhibition							
11:30–11:45	Poster Awards							
11:45–12:45	**Plenary Session VIII** *Food and feed microbiology*							
	p. 35							
12:45–13:00	Closing Remarks							

Short Lecture

CONFERENCE PROGRAMME

VAAM 2013 Jahrestagung Bremen (10.–13.03.2013)

Sunday, 10 March 2013

15:00–15:30	**Welcome Addresses** **Senatorin für Bildung und Wissenschaft** (angefragt) **Rudolf Amann** *Max-Planck-Institut für Marine Mikrobiologie, Bremen, Germany* **Gert-Jan Euverink** *Institute for Technology and Management, University of Groningen*	**Hanse-Saal**
	PUBLIC LECTURE **Chair: Matthias S. Ullrich**	**Hanse-Saal**
15:30–16:30	ISV01: **Helge Karch** *Universität Münster, Institut für Hygiene, Münster, Germany* EHEC-Bakterien – gestern und heute	
16:30–17:00	Coffee break/Industrial exhibition	**Hall 4.1**
	PLENARY SESSION I: MARINE MICROBIOLOGY **Chair: Rudolf Amann** **Co-Chair: Lucas Stal**	**Hanse-Saal**
17:00	ISV02: **Lars Peter Nielsen** *Aarhus University, Department of Biological Sciences, Aarhus, Denmark* Marine sediment hardwired by microbes	
17:30	ISV03: **Mikhail Zubkov** *National Oceanography Centre, Ocean Biogeochemistry & Ecosystems Research Group, Southampton, United Kingdom* Microbial uptake of depleted phosphate in the North Atlantic subtropical gyre	
	PLENARY SESSION II: UNICELLULAR EUKARYOTIC MICROBIOLOGY **Chair: Katja Metfies** **Co-Chair: Matthias S. Ullrich**	**Hanse-Saal**
18:00	ISV04: **E. Virginia Armbrust** *University of Washington, College of the Environment, School of Oceanography, Biological Oceanography, Seattle, WA, United States* Genomic insights into Phytoplankton capabilities	
18:30	ISV05: **Rene Wijffels** *Wageningen UR, Bioprocess Engineering, Wageningen, The Netherlands* Microalgal biotechnology	
19:00	Welcome Reception	Hall 4.1

Monday, 11 March 2013

08:00–19:30	Industrial exhibition	**Hall 4.1**
08:30–10:30	**Short lectures** (see page 42)	**various**
10:30–11:00	Coffee break/Industrial exhibition	**Hall 4.1**
	PLENARY SESSION III: HOST-MICROBE INTERACTION **Chair: Jetta Bijlsma** **Co-Chair: Barbara Reinhold-Hurek**	**Hanse-Saal**
11:00	**VAAM Honorary Award Session** ISO6: **Jens Boch** *Martin Luther University Halle-Wittenberg, Institute of Biology, Halle (Saale), Germany* A programmable DNA-binding domain	
11:45	ISV07: **Pierre de Wit** *Wageningen University, Laboratory of Phytopathology, Wageningen, The Netherlands* Comparative genomics of fungal plant pathogens and mechanisms of adaptation to their host plants	

CONFERENCE PROGRAMME

VAAM 2013 Jahrestagung Bremen (10.–13.03.2013)

12:15	ISV08: **Willem de Vos** *WU Agrotechnologie & Voedingswetenschappen, Laboratory of Microbiology, Wageningen, The Netherlands* Microbes inside – Interactions at the intestinal interface	
12:45–14:15	Lunch break/Industrial exhibition	**Hall 4.1**
14:15–18:00	**Mini-Symposium** **Special Group Quality Assurance and Diagnostics** (see page 36)	**Bruker Daltonik GmbH**
PLENARY SESSION IV: SINGLE CELL MICROBIOLOGY **Chair: Marcel Kuypers** **Co-Chair: Laura van Niftrik**		**Hanse-Saal**
14:15	ISV09: **Ramunas Stepanauskas** *Bigelow Laboratory for Ocean Sciences, Bigelow Laboratory Single Cell Genomics Center, East Boothbay, ME, United States* Individual look at microbes: Single cell genomics defies averages	
14:45	ISV10: **Laura van Niftrik** *Radboud University Nijmegen, Institute for Water and Wetland Research, Microbiology, Nijmegen, The Netherlands* Anammox bacteria: microbes with identity issues	
15:15–17:30	Coffee break/Industrial exhibition	**Hall 4.1**
15:15–17:30	Poster Session I (odd poster numbers)	**Hall 4.1 & Foyer**
15:30–17:00	**Karriere-Symposium** (see page 11)	**Kaisen-Saal**
17:30–19:30	**Special Groups Mini Symposia** (see page 36)	**various**
various	General Meetings of the Special Groups	**various**

Tuesday, 12 March 2013

08:00–19:00	Industrial exhibition	**Hall 4.1**
08:30–10:30	**Short lectures** (see page 45)	**various**
08:30–10:30	**Mini-Symposium Special Group Biotransformation** (see page 41)	**Salon Danzig**
10:30–11:00	Coffee break/Industrial exhibition	**Hall 4.1**
PLENARY SESSION V: OMICS AND BIOINFORMATICS **Chair: Frank Oliver Glöckner** **Co-Chair: Oscar Kuipers**		**Hanse-Saal**
11:00	ISV11: **Peer Bork** *EMBL Heidelberg, Heidelberg, Germany* Systemic analysis of human-associated microbes: Lessons from a tiny bacterium and a large community	
11:30	ISV12: **Kathleen Marchal** *Ghent University, Department of Plant Biotechnology and Bioinformatics, Ghent, Belgium* Network-based data integration for microbial systems biology	
PLENARY SESSION VI: ENVIRONMENTAL BIOTECHNOLOGY **Chair: Roland Benz** **Co-Chair: Mark van Loosdrecht**		**Hanse-Saal**
12:00	ISV13: **Marc Strous** *Max-Planck-Institut für Marine Mikrobiologie, Bremen, Germany* The end of the microbial redox tower?	

VAAM 2013 Jahrestagung Bremen (10.–13.03.2013)

12:30	ISV14: **Willy Verstraete** *LabMET, Ghent, Belgium* Microbial resources and their management: Where are we?	
13:00–14:30	**Industrial Lunch Symposium** (see page 14) **Pacific Biosciences**, Menlo Park, CA, United States	**Borgward-Saal**
13:00–14:30	Lunch break/Industrial exhibition	**Hall 4.1**
PLENARY SESSION VII: PHYSIOLOGY AND METABOLISM **Chair: Friedrich Widdel** **Co-Chair: Mike Jetten**		**Hanse-Saal**
14:30	ISV15: **Christa Schleper** *University of Vienna, Department of Genetics in Ecology, Vienna, Austria* Ammonia oxidizing Archaea: Physiology & evolution	
15:00	ISV16: **Mike S. M. Jetten** *Radboud University of Nijmegen, Faculty of Science, Dept. of Microbiology, Institute for Water and Wetland Research, The Netherlands* Discovery and physiology of "impossible" micro-organisms in Nitrogen and Methane Cycles	
15:30–17:30	**MIRRI-Symposium** (see page 48)	**Focke-Wulf-Saal**
15:30–17:30	Coffee break/Industrial exhibition	**Hall 4.1**
15:30–17:30	Poster Session II (even poster numbers)	**Hall 4.1 & Foyer**
17:00–18:15	**VAAM Annual General Meeting** (see page 12)	**Kaisen-Saal**
ca. 18:15	**PhD Awards** Sponsored by BASF SE, Sanofi Aventis Deutschland GmbH, Bayer Schering Pharma, New England Biolabs GmbH, Evonik Degussa GmbH	**Kaisen-Saal**
ca. 19:30	Mixer (see page 8)	**Weserstadion Bremen**

Wednesday, 13 March 2013

08:30–12:00	Industrial exhibition	**Hall 4.1**
09:00–11:00	**Short lectures** (see page 48)	**various**
11:00–11:30	Coffee break/Industrial exhibition	**Hall 4.1**
11:30–11:45	**Poster Awards** Sponsored by Bruker Daltonik GmbH	**Hanse-Saal**
PLENARY SESSION VIII: FOOD AND FEED MICROBIOLOGY **Chair: Marcel Zwietering** **Co-Chair: Tjakko Abee**		**Hanse-Saal**
11:45	ISV17: **Luc de Vuyst** *Vrije Universiteit Brussel VUB – IMDO, Faculty of Sciences and Bioengineering Sciences, Research Group of Industrial Microbiology and Food Biotechnology, Brussels, Belgium* Microbial ecology of the cocoa bean fermentation process	
12:15	ISV18: **Monika Ehling-Schulz** *University of Veterinary Medicine, Institute of Functional Microbiology Department of Pathobiology, Vienna, Austria* Emering spares: The Good, the Bad, the Ugly	
12:45–13:00	**Closing Remarks**	**Hanse-Saal**

ACTIVITIES OF THE SPECIAL GROUPS

Mini-Symposia of the Special Groups: Monday, March 11, 14:15–18:00

Special Group: Quality Assurance and Diagnostics (Qualitätssicherung und Diagnostik)

Topic: Maldi TOF and other modern identification techniques; lab visit and presentations

Organisation: S. Prowe, Beuth Hochschule für Technik, Fachbereich V – Studiengang Biotechnologie, Berlin, Germany; A. Seiffert-Störiko, Sanofi-Aventis Deutschland GmbH, Frankfurt-Höchst, Germany
Company site of Bruker Daltonik GmbH, Fahrenheitstraße 4, 28359 Bremen

14:15 **W. Pusch**
Bruker Daltonik GmbH, Bremen, Germany
Introduction to Bruker Daltonik GmbH

QDV1-FG 14:30 **M. Kostrzewa**
Bruker Daltonik GmbH, Molecular Biology R&D, Bremen, Germany
Introduction to microorganism analysis via MALDI-TOF

QDV2-FG 14:45 **A. W. Friedrich**
University Hospital Groningen, Department for Medical Microbiology and Infection Control, Groningen, The Netherlands
MALDI-TOF analytics for rapid identification of MRSA and other relevant species causing nosocomial infections

QDV3-FG 15:15 ***G. H. Wubbels, M. van der Wiel, T. Lijzenga , A. Douma, P. Willemse**
WLN, Glimmen, The Netherlands
The use of MALDI-TOF MS or (Q)RT-PCR of microbiological water analyses. A safety issue for the consumer

QDV4-FG 15:45 **I. Köhnen[1], S. Kupferer[1], F. Blessing[2], *M. Egert[1]**
[1]Hochschule Furtwangen University, Department of Mechanical and Process Engineering, Villingen-Schwenningen, Germany
[2]Group Practice for Laboratory Medicine, Singen, Germany
Scary meat? – Occurrence of antibiotic resistant bacteria (MRSA, ESBL) on conventional and organic retail meat samples in the greater area of Villingen-Schwenningen, Germany

16:15 Coffee break

16:30 Lab visit (in groups) and demonstration of the MALDI-TOF equipment, time for face-to-face discussions

18:00 End of Symposium

Mini-Symposia of the Special Groups: Monday, March 11, 17:30–19:30

Special Group VAAM: Environmental Microbiology (Umweltmikrobiologie)
Special Group KNVM: Microbial Ecology (Mikrobielle Ökologie)

Topic: Electron transfer processes in the subsurface

Organisation: R. U. Meckenstock, Helmholtz Zentrum München, Institute of Groundwater Ecology, München, Germany; L. J. Stal, University of Amsterdam, Royal Netherlands Institute of Sea Research (NIOZ) and Department of Aquatic Microbiology, Department of Marine Microbiology, Yerseke, The Netherlands
Kaisen-Saal

EMV1-FG 17:30 **B. Schink**
University of Konstanz, Microbial Ecology, Dept. of Biology, Konstanz, Germany
Interspecies electron transfer in lake sediments

EMV2-FG 18:00 **S. Y. Malkin, D. Seitaj, E.-M. Zetsche, D. Vasquez, H. T. S. Boschker, *F. J. R. Meysman**
Netherlands Institute of Sea Research (NIOZ), Yerseke, The Netherlands
Microbial batteries in the seafloor: Sulphide oxidation via long-distance electron transport

EMV3-FG 18:30 ***A. Piepenbrock, A. Kappler**
University of Tübingen, Center for Applied Geosciences, Tübingen, Germany
Humic substance reduction and electron transfer to Fe(III) minerals under environmental conditions

Mini-Symposia of the Special Groups: Monday, March 11, 17:30–19:30

EMV4-FG 18:42 ***R. Schauer[1,2], S. Larsen[1], K. U. Kjeldsen[1], J. J. Berg[2], L. Schreiber[1], N. Risgaard-Petersen[1], A. Schramm[1,2], L. P. Nielsen[1,2]**
[1]Aarhus University, Department of Bioscience, Center for Geomicrobiology, Aarhus, Denmark
[2]Aarhus University, Department of Bioscience, Microbiology, Aarhus, Denmark
Hunting for microbial generators of electric currents in marine sediments

EMV5-FG 18:54 ***D. Vasquez-Cardenas[1,2], R. Schauer[3], S. Hidalgo[2], S. Atli[1], V. Confurius[1], F. J. R. Meysman[2], H. T. S. Boschker[1]**
[1]Netherlands Institute of Sea Research (NIOZ), Marine Microbiology Department, Yerseke, The Netherlands
[2]Netherlands Institute of Sea Research (NIOZ), Ecosystem Studies Department, Yerseke, The Netherlands
[3]Aarhus University, Department of Bioscience, Center of Geomicrobiology, Aarhus, Denmark
Chemotrophy associated with microbial sulphide oxidation by long-distance electron transport in coastal sediment

EMV6-FG 19:06 ***M. Visser[1], P. Worm[1], P. Schaap[2], C. M. Plugge[1], A. J. M. Stams[1,3]**
[1]Wageningen University, Laboratory of Microbiology, Wageningen, The Netherlands
[2]Wageningen University, Laboratory of Systems and Synthetic Biology, Wageningen, The Netherlands
[3]University of Minho, Centre of Biological Engineering, Braga, The Netherlands
Key factors in syntrophy: Genome comparison between the syntroph *Pelotomaculum thermopropionicum* and the non-syntroph *Desulfotomaculum kuznetsovii*

EMV7-FG 19:18 ***H. Müller[1], J. Bosch[1], T. Lüders[1], L. R. Damgaard[2], L. P. Nielsen[2], R. U. Meckenstock[1]**
[1]Helmholtz Zentrum München, Institute of Groundwater Ecology, München, Germany
[2]Aarhus University, Department of Bioscience, Aarhus, Denmark
Long distance electron transfer in groundwater

Special Group: Regulation and Signal Transduction in Prokaryotes (Regulation und Signaltransduktion)

Topic: Protein modifications in gene regulation and signal transduction

Organisation: T. Mascher, Ludwig-Maximilians-University (LMU) Munich, Department Biology I, Microbiology, Planegg-Martinsried, Germany
Borgward-Saal

RSV1-FG 17:30 **I. Mijakovic**
AgroParisTech-INRA, MICALIS UMR 1319, Thiverval-Grignon, France
Bacterial phosphorylation networks: the systems biology perspective

RSV2-FG 18:00 **K. Turgay**
Leibniz Universität Hannover, Institut für Mikrobiologie, Hannover, Germany
Protein arginine phosphorylation in *Bacillus subtilis*

RSV3-FG 18:30 ***A. J. Wolfe[1], B. Zemaitaitis[1], B. Schilling[2], M. Kuhn[3], L. Hu[1], B. Lima[1], M. Scholle[3], M. Mrksich[3], W. Anderson[3], B. Gibson[2]**
[1]Loyola University Medical School, Department of Microbiology and Immunology, Maywood, IL, United States
[2]Buck Institute for Research on Aging, Novato, CA, United States
[3]Northwestern University, Molecular Pharmacology and Biological Chemistry, Feinberg School of Medicine, Chicago, IL, United States
Acetyl phosphate is a potent regulator of bacterial protein acetylation

RSV4-FG 19:00 **B. Khanh Chi[1], G. J. Palm[1], K. Bäsell[1], D. Becher[1], A. Roberts[2], C. J. Hamilton[2], *H. Antelmann[1]**
[1]Ernst-Moritz-Arndt-University of Greifswald, Institute for Microbiology, Greifswald, Germany
[2]University of East Anglia, School of Pharmacy, Norwich, United Kingdom
Protein *S*-bacillithiolation as reversible redox-switch mechanism in *Firmicutes* bacteria

Followed by **Annual Meeting of the Special Group "Regulation and Signal Transduction in Prokaryotes"**

ACTIVITIES OF THE SPECIAL GROUPS

Mini-Symposia of the Special Groups: Monday, March 11, 17:30–19:30

Special Group: Microbial Pathogenicity (Mikrobielle Pathogenität)

Topic: Pathogen adaptation to host organisms

Organisation: T. Mascher, Ludwig-Maximilians-University (LMU) Munich, Department Biology I, Microbiology, Planegg-Martinsried, Germany
Focke-Wulf-Saal

MPV1-FG 17:30 ***J. Seele[1], A. Singpiel[1], C. Spoerry[2], U. von Pawel-Rammingen[2], P. Valentin-Weigand[1], C. G. Baums[1]**
[1]University of Veterinary Medicine Hannover, Institute for Microbiology, Department of Infectious Diseases, Hannover, Germany
[2]Umeå University, Department of Molecular Biology and Umeå Centre for Microbial Research, Umeå, Sweden
Identification of a novel host-specific IgM protease in *Streptococcus suis*

MPV2-FG 17:45 ***S. Weber, H. Hilbi**
Ludwig-Maximilians Universität München, Max von Pettenkofer Institute, Munich, Germany
High resolution real-time imaging reveals a dynamic phosphoinositide pattern on the *Legionella*-containing vacuole

MPV3-FG 18:00 ***M. Sand, B. Averhoff**
Goethe University Frankfurt, Mol. Microbiology & Bioenergetics, Frankfurt, Germany
Mannitol, a compatible solute synthesized by *Acinetobacter baylyi* in a two-step-mechanism including a salt-induced and salt-dependent mannitol-1-phosphate dehydrogenase

MPV4-FG 18:15 ***M. M. Heimesaat[1], L.-M. Haag[1], A. Fischer[1], B. Otto[1], U. Grundmann[1], A. A. Kühl[2], U. B. Göbel[1], S. Bereswill[1]**
[1]Charité – Universitätsmedizin Berlin, Institut für Mikrobiologie und Hygiene, Berlin, Germany
[2]Charité – Universitätsmedizin Berlin, Dept. of Pathology Research Center ImmunoSciences (RCIS), Berlin, Germany
Campylobacter jejuni infection of infant mice: acute enterocolitis is followed by asymptomatic intestinal and extra-intestinal immune responses

MPV5-FG 18:30 ***L. Klingenbeck[1], R. A. Eckart[1], S. Bisle[1], C. Berens[2], A. Lührmann[1]**
[1]Friedrich-Alexander Universität Erlangen-Nürnberg, Universitätsklinikum Erlangen, Mikrobiologisches Institut, Erlangen, Germany
[2]Friedrich-Alexander Universität Erlangen-Nürnberg, Department Biologie, Erlangen, Germany
Coxiella burnetii harbours several anti-apoptotic type IV secretion system substrates with distinct molecular activities

MPV6-FG 18:45 ***A. Pelzer[1], H. Funken[1], T. Polen[2], S. Wilhelm[1], F. Rosenau[3], K.-E. Jaeger[1]**
[1]Heinrich-Heine-Universität Düsseldorf, Forschungszentrum Jülich, Institute of Molecular Enzyme Technology, Jülich, Germany
[2]Forschungszentrum Jülich, Institute of Bio- and Geosciences-1, Jülich, Germany
[3]Universität Ulm, Institute of Pharmaceutical Biotechnology, Ulm, Germany
Subtilase SprP regulates virulence phenotypes in *Pseudomonas aeruginosa*

MPV7-FG 19:00 ***B. Krismer[1], M. Liebeke[2], D. Janek[1], M. Rautenberg[1], G. Hornig[1], C. Weidenmaier[1], M. Lalk[2], A. Peschel[2]**
[1]University of Tübingen, Interfaculty Institute of Microbiology and Infection Medicine (IMIT), Tübingen, Germany
[2]University of Greifswald, Institute of Pharmacy, Greifswald, Germany
Metabolic adaptation of *Staphylococcus aureus* to nasal colonization reveals new antimicrobial target

MPV8-FG 19:15 ***L. Mahler[1], A. Otto[1], K. Dörries[2], S. Fuchs[1], D. Becher[1], M. Lalk[2], F. Schmidt[3], S. Monecke[4], M. Hecker[1], S. Engelmann[1]**
[1]University of Greifswald, Institute for Microbiology, Greifswald, Germany
[2]University of Greifswald, Institute for Biochemistry, Greifswald, Germany
[3]University of Greifswald, Interfaculty Institute for Genetics and Functional Genomics, Greifswald, Germany
[4]Alere Technologies, Jena, Germany
Adaptation of *Staphylococcus aureus* to human nasal secretions

Mini-Symposia of the Special Groups: Monday, March 11, 17:30–19:30

Special Group: Symbiotic Interactions (Symbiotische Interaktionen)

Topic: Microbial ecology of mice and man

Organisation: A. Schwiertz, Institut für Mikroökologie, Herborn, Germany; U. Hentschel Humeida, Universität Würzburg, Julius-von-Sachs Institut für Biowissenschaften, Würzburg, Germany
Lloyd-Saal

SIV1-FG 17:30 **H. Smidt**
Wageningen University, Agrotechnology & Food Sciences, Laboratory of Microbiology, Wageningen, The Netherlands
Monogastric model animals – Untangling the interplay of microbiota with the development of its mammalian host

SIV2-FG 18:00 **V. Martins dos Santos**
Wageningen University, Laboratory of Systems and Synthetic Biology, Wageningen, The Netherlands
Systems approaches to understand the interplay between gut microbiota and its mammalian host

SIV3-FG 18:20 ***A. Woting, G. Loh, M. Blaut**
German Institute of Human Nutrition (DIfE) Potsdam-Rehbrücke, Gastrointestinal Microbiology, Nuthetal, Germany
Obesity and the gut microbiota: the SIHUMI mouse model provides insights into relationships

SIV4-FG 18:35 ***M. Sadaghian, H. J. M. Harmsen**
UMCG, Medical microbiology, Groningen, The Netherlands
Localization of two major phylogroups of *Faecalibacterium prausnitzii* in human fecal samples

SIV5-FG 18:50 **H. Salem[1], S. Sudakaran[1], C. Kost[2], E. Kreutzer[3], *M. Kaltenpoth[1]**
[1]Max Planck Institute for Chemical Ecology, Insect Symbiosis Research Group, Jena, Germany
[2]Max Planck Institute for Chemical Ecology, Experimental Ecology and Evolution Research Group, Jena, Germany
[3]University of Regensburg, Department of Zoology, Regensburg, Germany
Actinobacteria as essential nutritional symbionts in pyrrhocorid bugs

SIV6-FG 19:05 ***D. Zhurina[1], J. Schützner[1], P. Walter[2], C. U. Riedel[1]**
[1]University Ulm, Institute of Microbiology and Biotechnology, Ulm, Germany
[2]University of Ulm, Central Electron Microscopy Facility, Ulm, Germany
Exploring Bifidobacterium bifidum S17 for potential players in host-microbe interactions by genomic and proteomic approaches

SIV7-FG 19:20 ***A. Probst, A. Auerbach, C. Moissl-Eichinger**
University of Regensburg, Department for Microbiology and Archaea Center, Regensburg, Germany
Thaumarchaeota on human skin – Implications for medical surveys?

Special Group: Biotransformation (Biotransformationen) together with/gemeinsam mit der AG Biokatalyse der Dechema

Topic: Microbes and catalytic biofilms: Industrial catalysts of the future?

Organisation: A. Schmid, K. Bühler, TU Dortmund University, Dept. Chemical and Biochemical Engineering, Laboratory of Chemical Biotechnology, Dortmund
Salon Danzig

BIOV1-FG 17:30 ***S. Molin[1,2], A. Toftgaard Nielsen[2], C. Sternberg[1]**
[1]Technical University of Denmark, Department of Systems Biology, Center for Systems Microbiology, Lyngby, Denmark
[2]Technical University of Denmark, Novo Nordisk Foundation Center for Biosustainability, Hørsholm, Denmark
Biofilms for chemical production – An outlook

BIOV2-FG 18:00 **H.-C. Flemming**
University of Duisburg-Essen, Biofilm Centre, Essen, Germany
Life and death in biofilms

ACTIVITIES OF THE SPECIAL GROUPS

Mini-Symposia of the Special Groups: Monday, March 11, 17:30–19:30

BIOV3-FG 18:30 ***B. Halan, A. Schmid, K. Bühler**
TU Dortmund University, Dept. Chemical and Biochemical Engineering, Laboratory of Chemical Biotechnology, Dortmund
Catalytic biofilms: Linking EPS composition to solvent stress

BIOV4-FG 18:50 ***K. Muffler[1], C. Schlegel[1], C. Müller[2], N. Davoudi[2], I. Reichenbach[3], J.C. Aurich[3], C. Ziegler[2], R. Ulber[1]**
[1]University of Kaiserslautern, Department of Mechanical and Process Engineering, Institute of Bioprocess Engineering, Kaiserslautern, Germany
[2]University of Kaiserslautern, Department of Physics and Research Center OPTIMAS, Kaiserslautern, Germany
[3]University of Kaiserslautern, Department of Mechanical and Process Engineering, Institute for Manufacturing Technology and Production Systems, Kaiserslautern, Germany
Microstructured bioreactor surfaces as supports for catalytic biofilms

BIOV5-FG 19:10 ***J. M. Kisch[1], C. Utpatel[2], W. Streit[2], A. Liese[1]**
[1]Hamburg University of Technology, Institute of Technical Biocatalysis, Hamburg, Germany
[2]University of Hamburg, Department of Microbiology and Biotechnology, Hamburg, Germany
Biofilm growth inhibition on medical plastic materials using immobilized esterases and acylases

Special Group: Microbial Cell Biology (Mikrobielle Zellbiologie)
Special Group: Archaea (Archaea)
Topic: Microbial compartmentalization

Organisation: H. Engelhardt, Max-Planck-Institut für Biochemie, Martinsried, Germany
Salon Franzius

MCV1-FG 17:30 **J. A. Fuerst**
The University of Queensland, School of Chemistry and Molecular Biosciences, St.Lucia, Australia
Compartmentalization in *Planctomycetes* – Structure and function of cell complexity in bacteria

MCV2-FG 18:00 **D. Devos**
Ruprecht-Karls-Universität Heidelberg, Biowissenschaften, COS Heidelberg, Heidelberg, Germany
Three-dimensional reconstruction of bacteria with a complex endomembrane system

MCV3-FG 18:30 ***R. Rachel[1], H. Huber[2], T. Heimerl[1], J. Flechsler[1], L. Kreuter[2], S. Daxer[3], K. Parey[3], C. Ziegler[3], G. Wanner[4], R. Wirth[2]**
[1]University of Regensburg, Centre for Electron Microscopy, Regensburg, Germany
[2]University of Regensburg, Institute of Microbiology and Archaea Centre, Regensburg, Germany
[3]University of Regensburg, Institute for Biophysics and Physical Biochemistry, Regensburg, Germany
[4]Universität München, Dept. Biology I – Botany, Planegg, Germany
Ignicoccus: Physiology, complexity and compartmentalization in a hyperthermophilic crenarchaeon

MCV4-FG 19:00 **H. Engelhardt**
Max-Planck-Institut für Biochemie, Martinsried, Germany
Concept of compartmentation (not only) in microbes

Followed by **Annual Meeting of the Special Group "Microbial Cell Biology"**

Special Group: Identification and Systematics (Identifizierung und Systematik)
Topic: Challenges in microbial identification

Organisation: H.-J. Busse, Veterinärmedizinische Universität, Institut für Bakteriologie, Mykologie und Hygiene, Vienna, Austria; C. M. Plugge, Wageningen University, Laboratory of Microbiology, Wageningen, The Netherlands
Salon London

SYV1-FG 17:30 ***J. S. Bennett, K. A. Jolley, M. C. J. Maiden**
University of Oxford, Department of Zoology, Oxford, Germany
Characterizing *Neisseria* species using Ribosomal Multilocus Sequence Typing

Mini-Symposia of the Special Groups: Monday, March 11, 17:30–19:30

SYV2-FG 18:00 ***H. Christensen[1], N. Nørskov-Lauritsen[2], J. J. de Witt[3], C. Hess[3], M. Bisgaard[4]**
[1]University of Copenhagen, Department of Veterinary Disease Biology, Section of Microbiology, Frederiksberg C, Denmark
[2]Aarhus University Hospital Skejby, Department of Clinical Microbiology, Skejby, Denmark
[3]GD B.V., Deventer, Netherlands
[4]University for Veterinary Medicine Vienna (Vetmeduni Vienna), Clinic for Avian, Reptile and Fish Medicine, Department for Farm Animals and Veterinary Public Health, Vienna, Austria
Importance of MLSA for classification of representatives of the genus *Avibacterium*

SYV3-FG 18:30 ***R. Rosselló-Móra[1], J. Tamames[2], E. Moore[3], M. Richter[4], P. Yarza[4], P. Schmitt-Kopplin[5]**
[1]IMEDEA, Ecology and Marine Resources, Esporles, Spain
[2]Centro Nacional de Biotecnología (CSIC), Madrid, Spain
[3]Culture Collection University of Gothenburg (CCUG), Gothenburg, Sweden
[4]Ribocon GmbH, Bremen, Germany
[5]Helmholtz Zentrum München, Neuherberg, Germany
New approaches to improve the bacterial species definition (or 'identification')

Optional **Annual Meeting of the Special Group "Identification and Systematics"**

Mini-Symposia of the Special Groups: Tuesday, March 12, 08:30–10:30

Special Group: Biotransformation (Biotransformationen) together with/gemeinsam mit der AG Biokatalyse der Dechema

Topic: Microbes and catalytic biofilms: Industrial catalysts of the future?

Organisation: A. Schmid, K. Bühler, TU Dortmund University, Dept. Chemical and Biochemical Engineering, Laboratory of Chemical Biotechnology, Dortmund
Salon Danzig

BIOV6-FG 08:30 **J. Eck**
B • R • A • I • N Aktiengesellschaft, Zwingenberg; Germany
Engineering biology: From biodiversity to "Designer Bugs"

BIOV7-FG 09:00 ***M. van Loosdrecht, R. Kleerebezem, Y. Lin, M. Winkler**
Delft University of Technology, Dept. of Biotechnology, Environmental Biotechnology, Delft, The Netherlands
The microbial ecology of aerobic granular sludge

BIOV8-FG 09:30 **J. Gescher**
Karlsruhe Institute of Technology (KIT), Institute for Applied Sciences, Karlsruhe, Germany
Applied respiration: What dissimilatory iron reducers and electrolithotrophs can offer applied sciences

BIOV9-FG 10:00 **F. Harnisch**
UFZ – Helmholtz Centre for Environmental Research, Department of Environmental Microbiology, Leipzig, Germany
Electrifying white biotechnology: Microbial bioelectrocatalysis & electrochemical steered fermentation

Monday, March 11, 08:30–10:30

Physiology I

Kaisen-Saal

Chair: Marc Strous

PHYV001
08:30
*S. MAROZAVA, W.F.M. RÖLING, J. SEIFERT, R. KÜFFNER, M. VON BERGEN, R.U. MECKENSTOCK
Insights into the physiology of *Geobacter metallireducens* at low growth rates

PHYV002
08:45
*S. LAHME, K. TRAUTWEIN, L. WÖHLBRAND, R. RABUS
Anaerobic growth of aromatic compound-degrading bacteria with mixtures of succinate and benzoate: simultaneous or sequential utilization?

PHYV003
09:00
*B. KRAFT, H.E. TEGETMEYER, T.G. FERDELMAN, M. STROUS
Dinitrogen or ammonium? – A matter of speed

PHYV004
09:15
*L. RUSS, B. KARTAL, H.J.M. OP DEN CAMP, M.S.M. JETTEN
Interactions of sulfur and nitrogen cycle bacteria in a marine, laboratory scale model system

PHYV005
09:30
*B. ZHU, D. SPETH, F. LUESKEN, J. KELTJENS, M.S.M. JETTEN, K. ETTWIG
The role of archaea in nitrate-dependent anaerobic oxidation of methane

PHYV006
09:45
*B. NOWKA, H. DAIMS, E. SPIECK
Comparative oxidation kinetics of *Nitrospira* and *Nitrobacter* pure cultures

PHYV007
10:00
*S. HEINE, Y. LI, M. ENTIAN, K. SAUER N. FRANKENBERG-DINKEL
NO-induced dispersion of *Pseudomonas aeruginosa* biofilms is mediated by the phosphodiesterase NbdA

PHYV008
10:15
A. MÜLLER, N. LUPILOVA, *L.I. LEICHERT
Bleach turns a conserved *Escherichia coli* protein from a family with a diverse set of functions into a highly efficient chaperone

ISME Session (Marine microbes I)

Borgward-Saal

Chair: Jens Harder
Co-Chair: Matthias S. Ullrich

MMIV001
08:30
R. STOCKER
Spying on the lives of marine microbes: The power of direct observation

MMIV002
09:00
*M. SIMON, T. BRINKHOFF, B. WEMHEUER, H.-A. GIEBEL, C. BEARDSLEY, I. BAKENHUS, R. DANIEL, S. VOGET
Major role of photoheterotrophic and CO oxidizing *Roseobacter* RCA population in the ocean

MMIV003
09:15
*S. BILLERBECK, H.-A. GIEBEL, T. BRINKHOFF, C. BEARDSLEY, L. GRAM, W.H. JEFFREY, M. SIMON
Distribution of clusters of the *Roseobacter* clade in global oceans

MMIV004
09:30
*S. LAASS, J. KLEIN, M. HEISIG, M. ROHDE, P. TIELEN, D. JAHN
Regulation of anaerobic respiratory pathways in *Dinoroseobacter* shibae

MMIV005
09:45
*A. KAMP, J. KNAPPE, P. STIEF, D. DE BEER
Diatoms – widespread dissimilatory nitrate reducers with overlooked impact on the marine nitrogen cycle

MMIV006
10:00
*M. KÖNNEKE, W. MARTENS-HABBENA, E. HAMANN, T.G. FERDELMAN, S. LITTMANN, M.M.M. KUYPERS, D.A. STAHL
Co-assimilation of organic carbon by Nitrosopumilus maritimus suggests utilization of oceanic DOC by marine ammonia-oxidizing archaea

MMIV007
10:15
*R. HAHNKE, A. MANN, H. TEELING, C. BENNKE, B. FUCHS, R. AMANN, J. HARDER
Dilution cultivation yielded novel psychrophilic marine bacteria, representatives of the phytoplankton decomposing community

Archaea

Focke-Wulf-Saal

Chair: Roland Benz
Co-Chair: Friedrich Widdel

ARV001
08:30
*M. KÜHNER, G. LAYER
Characterization of the alternative heme synthase Ahb-NirJ1 from *Methanosarcina barkeri*

ARV002
08:45
*C. BERG, V. VANDIEKEN, B. THAMDRUP, K. JÜRGENS
Significance of archaeal nitrification in hypoxic waters of the Baltic Sea

ARV003
09:00
*V. HEINZ, E. GAGEN, S. DAXER, M. KÖNNEKE, K.-U. HINRICHS, M. THOMM, R. RACHEL
***Nitrosopumilus maritimus*: analysis of its ultrastructure by electron microscopy**

ARV004
09:15
*A. KLINGL, R. HENNEBERGER, C. MOISSL-EICHINGER, H. HUBER, R. RACHEL
S-layer proteins in pyrite-oxidizing Bacteria and Archaea – Structure, function and its limited application as taxonomic marker

ARV005
09:30
*M. VAN WOLFEREN, K. HEINRICH, M. AJON, A.J.M. DRIESSEN, S.-V. ALBERS
Intraspecies aggregation of *Sulfolobus* cells mediated by UV-induced pili

ARV006
09:45
*R. WIRTH, M. MORA
A systematic study of the swimming behaviour of various species of the archaeal genus *Thermococcus*

Monday, March 11, 08:30–10:30

ARV007
10:00
*A. BELLACK, G. WANNER, E.H. EGELMAN, R. RACHEL, R. WIRTH
Ultrastructural characterization of the hyperthermophilic Archaeon *Methanocaldococcus villosus*

ARV008
10:15
*D. PETRASCH, A. KLETZIN
Growth by Anaerobic Sulfur Disproportionation in Acidianus ambivalens / Sulfurisphaera MC1 coculture

Omics for biodiversity, biotechnology and bioinformatics research

Lloyd-Saal

Chair: Frank Oliver Glöckner
Co-Chair: Oscar Kuipers

GMV001
08:30
*H. TEELING, B. FUCHS, J. HARDER, G. GERDTS, A. WICHELS, D. BECHER, T. SCHWEDER, G. MICHEL, M. CZJZEK, W. WILLATS, F.O. GLÖCKNER, R. AMANN
The MIMAS project and beyond: A collaborative effort for gaining detailed insights into carbohydrate-degrading marine bacteria with a focus on algal blooms in the German bight

GMV002
08:45
*L. SAYAVEDRA, J. PETERSEN, R. PONNUDURAI, M. KLEINER, M. RICHTER, S. WETZEL, E. PELLETIER, V. BARBE, T. SCHWEDER, S. MARKERT, N. DUBILIER
An arsenal of toxins in the genome of symbionts from deep-sea hydrothermal vent mussels

GMV003
09:00
*A. FERNANDEZ-GUERRA, A. BARBERÁN, R. KOTTMANN, F.O. GLÖCKNER, E.O. CASAMAYOR
Unraveling the unknowns in the metagenomic protein universe using Graphical Models

GMV004
09:15
*A. SARKAR, F. MACHT, B. REINHOLD-HUREK
Expression profile analysis of oxygen response in the nitrogen-fixing endophyte *Azoarcus sp.* bh72 by genome-wide DNA microarray

GMV005
09:30
*C.-E. WEGNER, W. LIESACK
Metatranscriptomic analysis of rice straw degradation by paddy soil microbial communities

GMV006
09:45
A. LABES
Genome based methods for the exploration of natural products from marine fungi for the treatment of cancer

GMV007
10:00
*U. BRANDT, J.H. WÜBBELER, S. HIESSL, J. SCHULDES, A. THÜRMER, R. DANIEL, A. STEINBÜCHEL
Sequencing and annotation of the genome of the mercaptosuccinate utilizing proteobacterium *Variovorax paradoxus* strain B4

GMV008
10:15
*M.H. MEDEMA, K. BLIN, P. CIMERMANCIC, P. ZAKRZEWSKI, Y. PAALVAST, M. FISCHBACH, T. WEBER, R. BREITLING, E. TAKANO
A computational perspective on microbial secondary metabolite biosynthesis

Biochemistry: Structure, pathway, and compounds

Salon Danzig

Chair: Karl-Heinz Blotevogel
Co-Chair: Ulrich Fischer

FTV001
08:30
*J. POPPE, R. HÜLSKÖTTER, B. KOWALEWSKI, K. SCHNEIDER, U. ERMLER
The Molybdenum Storage Protein – a special kind of metalloprotein

FTV002
08:45
*I. SECCARECCIA, M. NETT
Metallophores as predation factors in C*upriavidus necator*

FTV003
09:00
*A. MORGENSTERN, A. BEHREND, D. SPITELLER
Seeing more than red – Unexpected prodigiosin pigments in *Streptomyces coelicolor*

FTV004
09:15
*C. LANGE, J. KRAUSZE, J. REBELEIN, W.-D. SCHUBERT, M. RIBBE, D.W. HEINZ, J. MOSER, D. JAHN
Biosynthesis of Chlorophylls: Three-dimensional structure of ADP • AlF_3-stabilized protochlorophyllide oxidoreductase complex

FTV005
09:30
*U. BERTSCHE, T. ROTH, J. DEIBERT, S. REICHERT, D. KÜHNER
Deletion of *rodA* affects peptidoglycan composition in *Staphylococcus carnosus* TM300

FTV006
09:45
*F. BUERMANN, H.-C. SHIN, J. BASQUIN, B.-H. OH, *S. GRUBER
Architecture of Smc–ScpAB

FTV007
10:00
*P. CUNHA TAROUCO, H. MOUTTAKI, R.U. MECKENSTOCK
Carboxylation is a common biochemical strategy to activate naphthalene in several anaerobic bacteria

FTV008
10:15
*B. SEIP, D. THAKRAL, M. KURZ, E.A. GALINSKI, C.A. INNIS, T.A. STEITZ
Impact of the compatible solute hydroxyectoine on cell-free protein synthesis

Bacterial signal transduction: From individual genes to multicellular communities

Salon Franzius

Chair: Georgi Muskhelishvili
Co-Chair: Helge Weingart

GRV001
08:30
*K.T. KOVÁCS, T. SPALDING, A. ROVETTO, R. GRAU, O.P. KUIPERS
Living on the surface: Biofilm formation and surface motility of *Bacillus subtilis*

GRV002
08:45
*A. HEYER, A.M. NANDA, A. GRÜNBERGER, D. KOHLHEYER, J. FRUNZKE
Spontaneous prophage induction in *Corynebacterium glutamicum*

Monday, March 11, 08:30–10:30

GRV003
09:00
S. DINTNER, F. KALLENBERG, *S. GEBHARD
Interactions between a sensor kinase and an ABC-transporter: a new signalling pathway in antimicrobial peptide detoxification modules

GRV004
09:15
*S. GRAF, G. UNDEN
Interaction of the fumarate sensor DctS with the binding protein DctB in *B. subtilis*

GRV005
09:30
*S. BRAMEYER, A.O. BRACHMANN, Y. KOPP, H. JANOSZ, H.B. BODE, R. HEERMANN
The PpyS/PluR system of *Photorhabdus luminescens* mediates a novel way for cell-cell communication using pyrones

GRV006
09:45
*M. MCINTOSH, J. SERRANIA, A. BECKER
Intricate regulatory circuitry ensures the dependence of quorum sensing on nutrient limitation AND population density

GRV007
10:00
*K. MORABBI HERAVI, J. ALTENBUCHNER
Transcription activation of the promoters of mannitol utilization system in *Bacillus subtilis*

GRV008
10:15
*B. BERGHOFF, A. KONZER, N. MANK, M. LOOSO, T. RISCHE, M. KRÜGER, G. KLUG
Integrative „Omics"-Approach discovers dynamic and regulatory features of the bacterial response to singlet oxygen

Structural and functional dynamics of the gut microbiota in interaction with the host

Salon London

Chair: Hermie J. M. Harmsen
Co-Chair: Barbara Reinhold-Hurek

GOMV001
08:30
S. PANDE, H. MERKER, K. BOHL, M. REICHELT, A. LÜCK, L. DE FIGUEIREDO, S. GERMERODT, S. SCHUSTER, C. KALETA, *C. KOST
Less is more – Bacterial gene loss results in a division of metabolic labour and the formation of intercellular networks

GOMV002
08:45
*J. SEIFERT, S.-B. HAANGE, A. OBERBACH, A. RUIZ, A. SUAREZ, M. FERRER, M. VON BERGEN
Functional microbial ecology in the gut ecosystem revealed by metaproteomics– from feces to the mucus layer

GOMV003
09:00
*C. SCHWAB, D. BERRY, I. RENNISCH, THE INFLAMMOBIOTA CONSORTIUM C. SCHLEPER, A. LOY, T. URICH
Lasting impact of acute inflammation on murine intestinal microbiota structure and function

GOMV004
09:15
*E. MANN, S. SCHMITZ-ESSER, Q. ZEBELI, M. WAGNER, M. RITZMANN, B. METZLER-ZEBELI
Deep sequencing reveals promotion of gastric lactobacilli in weaned pigs by increased dietary calcium-phosphorus levels

GOMV005
09:30
*J. BUDNOWSKI, L. HANSKE, S. SCHLECHTE, M. BLAUT
Bacterial activation of glucosinolates: Variety is the spice of life

GOMV009
09:45
*T. BELLO GONZALEZ, P. PHAM, M. VAN PASSEL, H. SMIDT
Survival traits of *Enterococcus spp.* in the human gut

GOMV007
10:00
*M.M. HEIMESAAT, L.-M. HAAG, A. FISCHER, B. OTTO, R. PLICKERT, A.A. KÜHL, U.B. GÖBEL, S. BERESWILL
Intestinal microbiota shifts towards elevated commensal *Escherichia coli* loads abrogate colonization resistance against *Campylobacter jejuni* in mice

GOMV008
10:15
*C. SCHRÖDER, A. MATTHIES, W. ENGST, M. BLAUT, A. BRAUNE
Identification and functional expression of isoflavone conversion genes from the human intestinal bacterium *Slackia isoflavoniconvertens*

Food, feed, fuel – Microbes in action

Salon Bergen

Chair: Gert-Jan Euverink
Co-Chair: Bernd Mahro

AMV001
08:30
*M. NOLL, S. AL DAHOUK
Antibiotic resistances panel of 336 *Listeria monocytogenes* strains isolated from German food and patient samples

AMV002
08:45
*S. SCHMITZ-ESSER, A. MÜLLER, M. MUHTEREM-UYAR, A. ZAISER, B. STESSL, K. RYCHLI, M. WAGNER
Tn6188 – a novel transposon in *Listeria monocytogenes* conferring tolerance to benzalkonium chloride

AMV003
09:00
*P. MESTER, D. SCHODER, M. WAGNER, P. ROSSMANITH
Matrix Lysis: A sample preparation method for recovery of bacterial targets based on solubilization of the sample matrix.

AMV004
09:15
*Y.S. KIM, P. SCHERER
Rapid quantification and classification of bacteria and methanogens by digital image analysis

Monday, March 11, 08:30–10:30

AMV005
09:30
*A. SCHMIDT, N. MÜLLER, B. SCHINK, D. SCHLEHECK
A proteomic view at the biochemistry of syntrophic butyrate oxidation in *Syntrophomonas wolfei*

AMV006
09:45
*F. GOLITSCH, C. BÜCKING, J. GESCHER
Proof of principle for a microbial fuel cell biosensor based on *Shewanella oneidensis* outer membrane protein complexes

AMV007
10:00
*S. HETZLER, A. STEINBÜCHEL
Production of lipids from lignocellulose with recombinant *R. opacus* PD630 strains

AMV008
10:15
*P. TIMMERS, J. GIETELING, A. WIDJAJA-GREEFKES, C. PLUGGE, A. STAMS, P. LENS, R. MEULEPAS
Novel high-pressure membrane-capsule bioreactor for the enrichment of anaerobic methanotrophs

Tuesday, March 12, 08:30–10:30

Physiology II

Kaisen-Saal

Chair: Jens Harder

PHYV009
08:30
*S. HIESSL, R. VIVOD, J. RABE, S. OETERMANN, A. STEINBÜCHEL
Microbial degradation of rubber by *Gordonia polyisoprenivorans*

PHYV010
08:45
*G. SCHMITT, J. BIRKE, D. JENDROSSEK, E.-M. BURGER, O. EINSLE
The catalytic haem centre of extracellular rubber oxygenase RoxA is oxygenated

PHYV011
09:00
*J. HOLERT, O. YÜCEL, A. KULIĆ, H. MÖLLER, B. PHILIPP
Degradation of the acyl side chain of the steroid compound cholate in *Pseudomonas* sp. strain Chol1 proceeds via an aldehyde intermediate

PHYV012
09:15
*R. MOSER, M. AKTAS, C. FRITZ, F. NARBERHAUS
Novel phospholipid biosynthesis pathways identified in *Xanthomonas campestris*

PHYV013
09:30
*D. WETZEL, R.-J. FISCHER
Analysis of small acid-soluble spore proteins in *Clostridium acetobutylicum*

PHYV014
09:45
*D. FALKE, M. FISCHER, G. SAWERS
Oxygen and nitrate respiration in spores of *Streptomyces coelicolor*

PHYV015
10:00
*E. KARINOU, E.L.R. COMPTON, A. JAVELLE
The *Escherichia coli* SLC26 homologue YchM (DauA) is a C_4-dicarboxylic acid transporter

PHYV016
10:15
*S. ROMANO, H. SCHULZ-VOGT, V. BONDAREV
Drastic physiological and morphological variation in strain *Pseudovibrio* FO-BEG1 induced by phosphorus limitation

Bacterial cell biology

Borgward-Saal

Chair: Leendert Hamoen
Co-Chair: Laura van Niftrik

CBV001
08:30
*C. JOGLER, M. JOGLER, M. SCHÜLER
Towards understanding the planctomycetal cell biology

CBV002
08:45
*H. STRAHL, L.W. HAMOEN
The actin homolog MreB organizes the bacterial cell membrane

CBV003
09:00
*S. NEUMANN, M.S.M. JETTEN, L. VAN NIFTRIK
The isolation of a 'prokaryotic cell organelle' from an anammox bacterium

CBV004
09:15
*K. JONAS, J. LIU, P. CHIEN, M.T. LAUB
Stress-induced protein misfolding arrests the *Caulobacter crescentus* cell cycle

CBV005
09:30
*D. PFEIFFER, D. JENDROSSEK
Attachment of PHB granules to the DNA is mediated via PhaM in *Ralstonia eutropha*

CBV006
09:45
D. PATZELT, H. WANG, I. BUCHHOLZ, M. ROHDE, L. GRÖBE, A. NEUMANN, S. SCHULZ, S. PRADELLA, R. MÜNCH, D. JAHN, I. WAGNER-DÖBLER, *J. TOMASCH
You are what you talk: Quorum sensing induces individualisation of the algal symbiont *Dinoroseobacter shibae* DFL-12

CBV007
10:00
*R. MERCIER, Y. KAWAI, P. DOMINGUEZ-CUEVAS, J. ERRINGTON
Excess membrane synthesis drives a primitive mode of cell proliferation

CBV008
10:15
*M. PILHOFER, G.J. JENSEN
Imaging intact bacterial cells in a life-like state, in three dimensions and to molecular resolution

Tuesday, March 12, 08:30–10:30

Geomicrobiology: From rocks to communities and genomes I

Focke-Wulf-Saal

Chairs: Michael W. Friedrich
Co-Chair: Marcel Kuypers

GEOV001
08:30
*J. MILUCKA, T.G. FERDELMAN, L. POLERECKY, D. FRANZKE, G. WEGENER, M. SCHMID, I. LIEBERWIRTH, M. WAGNER, F. WIDDEL, M.M.M. KUYPERS
Sulfur cycling associated with the anaerobic oxidation of methane

GEOV002
08:45
*V. VANDIEKEN, B. THAMDRUP
Hydrogen and acetate as electron donors for microbial manganese reduction in a manganese-rich marine sediment

GEOV003
09:00
*A. DREIER, L. STANNEK, M. TAVIANI, M. BLUMENBERG, M. SIGOVINI, C. WREDE, V. THIEL, M. HOPPERT
Signatures of endosymbiosis in recent and fossil bivalves

GEOV004
09:15
*L. VILLANUEVA, N. BALE, Y. LIPSEWERS, E. HOPMANS, S. SCHOUTEN, J. SINNINGHE DAMSTÉ
Tracing seasonal and spatial diversity and activity of marine Thaumarchaeota using intact polar lipids and gene expression

GEOV005
09:30
*F. BEULIG, V. HEUER, D. AKOB, M. ELVERT, B. VIEHWEGER, K.-U. HINRICHS, K. KÜSEL
Cold volcanic CO_2 emanations in a wetland area promote *Acidobacteria*, acetogens and methanogens

GEOV006
09:45
*M. HERRMANN, S. OPITZ, A. RUSZNYAK, I. SCHULZE, D. AKOB, K.-U. TOTSCHE, K. KÜSEL
Elucidation of microbial communities involved in CO_2 fixation in karstic limestone aquifers targeting RubisCO- and ammonia-monooxygenase encoding genes

GEOV007
10:00
*S. GWOSDZ, I. MÖLLER, H.H. RICHNOW, M. KRÜGER
Natural CO_2 vents affect freshwater environment

GEOV008
10:15
*S. MOSLER, A. POEHLEIN, S. VOGET, R. DANIEL, J. KIPRY, M. SCHLÖMANN, M. MÜHLING
Predicting the metabolic potential of a novel mining associated iron oxidizing bacterium by comparative genomics

Microbes and their environments

Lloyd-Saal

Chair: Bernhard Fuchs
Co-Chair: Annelies Veraart

FTV009
08:30
*E. PRUESSE, J. PEPLIES, W. LUDWIG, R. WESTRAM, F.O. GLÖCKNER
ARB phylogenetic sequence analysis suite – A preview on version 6

FTV010
08:45
*D. DE MAEYER, J. RENKENS, L. DE RAEDT, K. MARCHAL
Interaction networks to analyze gene lists from omics data

FTV011
09:00
A. NEMES, S. GRUBER, M. MARCHETTI-DESCHMANN, *S. ZEILINGER
MAPK signaling and mycoparasitism: Transcriptomic and proteomic approaches to dissect the mycoparasitic interaction of the biocontrol fungus *Trichoderma* with phytopathogenic host fungi

FTV012
09:15
*G. LENTENDU, A. CHATZINOTAS, T. WUBET, F. BUSCOT, C. WILHELM, M. SCHLEGEL
Soil eukaryotic unicellular microorganisms facing crop fertilization: A metagenomic approach

FTV013
09:30
*A. VERAART, E. FAASSEN, V. DAKOS, E. VAN NES, M. LÜRLING, M. SCHEFFER
Microbes under stress – Slowing down of population recovery as a warning for an approaching collapse

FTV014
09:45
*A. KOCH-KOERFGES, H.M. WOO, M. BOTT
Absence of cytochrome aa_3 oxidase causes oxidative stress in *Corynebacterium glutamicum*

FTV015
10:00
*C. WENTRUP, A. WENDEBERG, M. SCHIMAK, J. HUANG, C. BOROWSKI, N. DUBILIER
Symbiont colonization occurs throughout the entire life cycle of hydrothermal vent mussels

FTV016
10:15
*S.L. GARCIA, F. WARNECKE
Freshwater actinobacteria: From uncultivated to enigmatic

Polar microbial ecology/ Marine microbes II

Salon Franzius

Chair: Antje Boetius
Co-Chair: Katja Metfies

PMEV001
08:30
*M.A. HORN, K. PALMER
Unexpected microbial drivers of N_2O fluxes in arctic permafrost-affected peatlands

PMEV002
08:45
*A. GITTEL, J. BARTA, I. LACMANOVA, B. WILD, J. SCHNECKER, R. MIKUTTA, B. HANISDAL, S. OWENS, J. GILBERT, V. TORSVIK, G. GUGGENBERGER, A. RICHTER, C. SCHLEPER, T. URICH
Microbial communities associated with buried carbon in cryoturbated soils of the Siberian Arctic and their response to a warming climate

PMEV003
09:00
*C. BIENHOLD, F. WENZHÖFER, A. BOETIUS
Benthic bacterial communities in a changing Arctic Ocean – a case study on the Laptev Sea margin (Arctic Ocean)

Tuesday, March 12, 08:30–10:30

PMEV004
09:15
*J. GÖRSCH, D. WAGNER
The El'gygytgyn Crater Lake – an example for the deep-biosphere in up to 3.6 million year old lake sediments

MMIV008
09:30
*S. THIELE, B.M. FUCHS, R.I. AMANN, M.H. IVERSEN
Bacterial distribution on marine now aggregates and its implication on the marine carbon cycle

MMIV009
09:45
*I. TORRES-MONROY, M.S. ULLRICH, A. STAHL
Identification of bacterial genes required for diatom-bacteria interactions during marine aggregate formation

MMIV010
10:00
*W. HAO, G. GERDTS, J. PEPLIES, A. WICHELS
Bacterial community associated with Ctenophores at Helgoland Roads, German Bight

MMIV011
10:15
*S. OBERBECKMANN, A.M. OSBORN
Plastic pollution and marine microbes: Exploring the plastic colonising community in the ocean

Dissecting virulence I

Salon London

Chair: Roland Benz
Co-Chair: Bernard A. M. van der Zeijst

MMAV001
08:30
*T. BOSCH, E. VERKADE, M. VAN LUIT, R. BURGGRAVE, J. KLUYTMANS, L. SCHOULS
High resolution typing of livestock-associated methicillin-resistant *Staphylococcus aureus* using whole genome mapping enables identification of transmission events

MMAV002
08:45
*C. LASSEK, M. BURGHARTZ, D. CHAVES-MORENO, B. HESSLING, A. OTTO, S. FUCHS, J. BERNHARDT, M. JAHN, D. BECHER, D. PIEPER, K. RIEDEL
Host-pathogen interactions during a catheter-associated urinary tract infection analysed by metaproteomics

MMAV003
09:00
W. BEHRENS, T. SCHWEINITZER, M. DORSCH, H.-J. HEDRICH, A. BLEICH, S. SUERBAUM, *C. JOSENHANS
Role of the proposed *Helicobacter pylori* energy sensor TlpD *in vivo* in the Mongolian gerbil model and whole genome analysis of a gerbil-adapted *H. pylori* strain

MMAV004
09:15
*U. MÄDER, P. NICOLAS, M. DEPKE, M. VAN DER KOOI-POL, J. PANÉ-FARRÉ, M. DEBARBOUILLE, C. GUERIN, A. HIRON, A. LEDUC, S. MICHALIK, E. REILMAN, F. SCHMIDT, P. NOIROT, P. BESSIÈRES, M. HECKER, T. MSADEK, U. VÖLKER, J.M. VAN DIJL
Whole-transcriptome analysis of Staphylococcus aureus under laboratory and infection-related conditions

MMAV005
09:30
*M. ABDULLAH, M. SALEH, N. GISCH, F. VOSS, L. PETRUSCHKA, T. KOHLER, T. PRIBYL, S. HAMMERSCHMIDT
Impact of a putative carboxypeptidase lipoprotein SPD_0549 on pneumococcal peptidoglycan synthesis and virulence

MMAV006
09:45
*B. SINGH, Y.-C. SU, M. MORGELIN, S. ANDERS, U. NILSSON, K. RIESBECK
***Haemophilus influenzae* surface fibril (Hsf) is a double folded trimeric autotransporter that binds two vitronectin molecules to enhance serum resistance**

MMAV007
10:00
*K. ZETH, C. SONG, C. WEICHBRODT, C. STEINEM, B.D. GROOT, U. ZACHARIAE
Crystal structure and functional mechanism of the human antimicrobial membrane channel dermcidin

MMAV008
10:15
V. WINSTEL, A. PESCHEL, *G. XIA
Unique *Staphylococcus aureus* wall teichoic acid prevents phage-mediated horizontal transfer of pathogenicity islands

Bacterial regulation and small non coding RNAs

Salon Bergen

Chairs: Barbara Reinhold-Hurek
Co-Chair: Georgi Muskhelishvili

BRV001
08:30
*C. PESAVENTO, M. ZIETEK, A. TYPAS
A signal transduction system tying cell shape with cell divisvion in *E. coli*

BRV002
08:45
*P. BEYERSMANN, M. BERGER, J. TOMASCH, I. WAGNER-DÖBLER, M. SIMON, T. BRINKHOFF
The antibiotic tropodithietic acid can replace acylated homoserine lactone as global gene regulator in *Phaeobacter* sp. DSM 17395

BRV003
09:00
*H. STEENACKERS, S. ROBIJNS, S. ROBERFROID, A. DE WEERDT, S. DE KEERSMAECKER, J. VAN DER LEYDEN
A GFP promoter fusion library for the study of *Salmonella* biofilm formation and the mode of action of biofilm inhibitors

BRV004
09:15
I.M. AXMANN
Cyanobacteria's specific features: Non-standard circadian clocks and plenty of antisense RNAs

BRV005
09:30
*Y. GÖPEL, K. PAPENFORT, B. REICHENBACH, J. VOGEL, B. GÖRKE
Targeted decay of small RNA GlmZ by RNase E adaptor protein YhbJ and inhibition by sRNA mimicry

BRV006
09:45
*S.R. PERNITZSCH, D. BEIER, C.M. SHARMA
A small RNA represses expression of the TlpB chemotaxis receptor by targeting a homopolymeric G-repeat

Tuesday, March 12, 08:30–10:30

BRV007
10:00
*D.P. PETROV, R. KRÄMER, G.M. SEIBOLD
Novel response on central metabolism perturbations in *Corynebacterium glutamicum*: Instantaneous stop of phosphotransferase system-mediated sugar uptake

BRV008
10:15
G. DUGAR, A. HERBIG, K. FÖRSTNER, N. HEIDRICH, R. REINHARDT, K. NIESELT, *C.M. SHARMA
High-resolution comparative transcriptome analysis of multiple *Campylobacter jejuni* strains

MIRRI-Symposium

Focke-Wulf-Saal

Chair: Frank Oliver Glöckner

MIRRI001
15:30
D. FRITZE
The pan-European initiative MIRRI: The Microbial Resource Research Infrastructure

MIRRI002
16:00
D. SMITH
The means to achieve the goals of the Microbial Resource Research Infrastructure (MIRRI)

MIRRI003
16:30
I. GILLESPIE
The pan-European Initiative MIRRI: Needs and impact

MIRRI004
17:00
*F.O. GLÖCKNER, A. KLINDWORTH
The pan-European Initiative MIRRI: Data resources management

Wednesday, March 13, 09:00–11:00

Biochemistry, Metabolism

Kaisen-Saal

Chair: Ralf Rabus

PHYV017
09:00
S. KELLER, M. RUETZ, B. KRÄUTLER, G. DIEKERT, *T. SCHUBERT
Guided *de novo* corrinoid biosynthesis of Benzimidazolyl-Norcobamides in the organohalide-respiring *Sulfurospirillum multivorans*

PHYV018
09:15
O. KLIMMEK
Respiratory complex iron-sulfur molybdoenzymes (CISM): Diversities and similarities of CISM with membrane-bound subunits of the PsrC/NrfD-type

PHYV019
09:30
*J. DERMER, J. EIPER, M. BOLL
Specific Mo-cofactor containing tertiary C-25 hydroxylases involved in anoxic degradation of steroids with variations in the side chain

PHYV020
09:45
*R. JARLING, M. SADEGHI, M. DROZDOWSKA, S. LAHME, A. GRUNER, W. BUCKEL, R. RABUS, F. WIDDEL, B.T. GOLDING, H. WILKES
Mechanistic and stereochemical investigations on the transformation pathways of hydrocarbons in *n*-alkane-degrading, anaerobic bacteria

PHYV021
10:00
*A. KLETZIN, A. VEITH, T. URICH, C. FRAZÃO, H.M. BOTELHO, C. GOMES
Structure/function relationship in sulfur oxygenase reductases from thermophilc archaea and mesophilic bactreria

PHYV022
10:15
*J. BERTSCH, V. MÜLLER
An electron bifurcating caffeyl-CoA reductase in the acetogenic bacterium *Acetobacterium woodii*

PHYV023
10:30
*K. BRANDT, D. MÜLLER, D. MATTHIES, T. MEIER, V. MÜLLER
Structure and function of the unique membrane-embedded rotor of the Na F_1F_O ATP synthase of *Acetobacterium woodii*

PHYV024
10:45
*K. KARSTENS, M. GRUNZEL, B. FRIEDRICH, O. LENZ
In search of an electron reservoir that protects the soluble, NAD -reducing [NiFe]-hydrogenase from detrimental effects of oxygen

Industrial biotechnology

Borgward-Saal

Chair: Michiel Kleerebezem
Co-Chair: Tilman Achstetter

IBV001
09:00
*E. NEVOIGT, J. MCINNES, M. CARRILLO, V. ZLATESKI, S. SWINNEN, M. KLEIN, H. THI THANH NGUYEN
Re-evaluation of glycerol utilization within the species *Saccharomyces cerevisiae*

IBV002
09:15
*A. GRÜNBERGER , N. MUSTAFI, C. PROBST, J. FRUNZKE , W. WIECHERT, D. KOHLHEYER
High-throughput microbial single cell analysis in picoliter bioreactors

IBV003
09:30
*M. BASEN, M.W.W. ADAMS
Engineering a hyperthermophilic archaeon for temperature-dependent gene expression and bioproduct formation

IBV004
09:45
*A. SCHMIDBERGER, M. HENKEL, U. OBST, R. HAUSMANN, T. SCHWARTZ
Iron-dependent regulation of rhamnolipid synthesis in *Pseudomonas aeruginosa* PAO1

IBV005
10:00
*G. SCHENDZIELORZ, S. BINDER, L. EGGELING, M. BOTT
Metabolitesensors for rapid screening and single-cell isolation of small-molecule producing bacteria

IBV006
10:15
*S.A.E. HEIDER, V.F. WENDISCH, P. PETERS-WENDISCH
Carotenoid production in *Corynebacterium glutamicum*

Wednesday, March 13, 09:00–11:00

IBV007
10:30
*J. MOCK, J. HEIDER
Synthetic microbial pathway for (*R*)-benzylsuccinate production

IBV008
10:45
*J. ÖHRLEIN, B. REINHOLD-HUREK, T. ACHSTETTER
Development of an online reporter system for plasmid stability in *Saccharomyces cerevisiae*

Proteomics and metabolomics – From communities to cells to pathways

Focke-Wulf-Saal

Chair: Jens Harder
Co-Chair: Jan Maarten van Dijl

PRMV001
09:00
*R. KERMER, T. WUBET, F. BUSCOT, M. VON BERGEN, J. SEIFERT
Metabolic networks and carbon and nitrogen flux in leaf litter degradation investigated by protein-stable isotope probing (protein-SIP)

PRMV002
09:15
*D. BENNDORF, R. KUHN, F. KOHRS, T. SCHLEGEL, L.L. PALESE, A. POLLICE, A. HANREICH, R. HEYER, R. KAUSMANN, M. HEIERMANN, M. KLOCKE, E. RAPP, U. REICHL
Metaproteomics for process monitoring in environmental biotechnology

PRMV003
09:30
*T. WEISSGERBER, M. SYLVESTER, V. GIESELMANN, C. DAHL
Quantitative proteomics of *Allochromatium vinosum*: insights into dissimilatory sulfur metabolism in a purple sulfur bacterium

PRMV004
09:45
N. RAATSCHEN, M. WENZEL, L. LEICHERT, U. KRÄMER, *J. BANDOW
Ionophores calcimycin and ionomycin extract iron and manganese from soil bacteria – A potent strategy against competitors

PRMV005
10:00
*N. MOLIERE, S. RUNDE, A. HEINZ, E. MAISONNEUVE, A. JANCZIKOWSKI, A. ELSHOLZ, U. GERTH, M. HECKER, K. TURGAY
Thermotolerance in *Bacillus subtilis*: The role of oxidation in survival and heat induced aggregate formation

PRMV006
10:15
*N. DE ALMEIDA, H. WESSELS, J. KELTJENS, M. JETTEN , B. KARTAL
Total membrane complexome profiling of the anammox bacterium *K. stuttgartiensis*

PRMV007
10:30
*F. TER VELD, D. WOLFF, C. SCHORSCH, T. KÖHLER, E. BOLES, A. POETSCH
Lipidomics and proteomics identifies oleaginous yeast-like behavior and two novel acetyltransferases in tetraacetyl phytosphingosine producing yeast Wickerhamomyces ciferrii

PRMV008
10:45
*A. KÜBERL, B. FRÄNZEL, D.A. WOLTERS, L. EGGELING, T. POLEN, M. BOTT
Pupylation in the biotechnological workhorse *Corynebacterium glutamicum*

Molecular ecology and techniques

Lloyd-Saal

Chair: Rudolf Amann
Co-Chair: Thomas Hurek

METV001
09:00
*P. YILMAZ, F.O. GLÖCKNER
Towards a unified taxonomy for ribosomal RNA databases

METV002
09:15
*P.K. WÜST, H. NACKE, K. KAISER, C. FISCHER, A. THÜRMER, B. FOESEL, D. BERNER, J. SIKORSKI, S. MARHAN, E. KANDELER, R. DANIEL, J. OVERMANN
Be aware of your nucleic acid extraction bias: insights from qPCR and deep sequencing of DNA and RNA extracts from soil

METV003
09:30
*T. YAMAGUCHI, S. KAWAKAMI, M. HATAMOTO, M. TAKAHASHI, K. KUBOTA, H. IMACHI, N. ARAKI, T. YAMAGUCHI
A novel detection method for visualizing environmental microbes with low rRNA content and low cell permeability by using *in situ* HCR-FISH

METV004
09:45
*M. WINKEL, P. PJEVAC, M. KLEINER, W. BACH, A. MEYERDIERKS, M. MUSSMANN
Evidence for heterotrophic microorganisms in diffuse fluids at two hydrothermal systems

METV005
10:00
*I. ADAM, F.-A. HERBST, J. SEIFERT, M. VON BERGEN, A. MILTNER, M. KÄSTNER
Compost gourmets – Using stable isotope probing for elucidating the carbon flux of the PAH degradation in composting environments and the responsible microorganisms

METV006
10:15
S.P. GLAESER, F. LEUNERT, K.U. FÖRSTNER, J. VOGEL, M. ALLGAIER, H.-P. GROSSART, *J. GLAESER
The response of *Polynucleobacter necessarius* to light in a humic lake

METV007
10:30
*H. SCHMIDT, T. EICKHORST
Microbes and root-soil interfaces: *in situ* analysis of single cells in rhizosphere research

METV008
10:45
*H.-C. SELINKA, H. DIZER, C. MEKONNEN, C. ARNDT, B. SÜSSENBACH, C. GÖTZ, I. GRÄBER, N. HARTMANN, A. FROHNERT, R. SZEWZYK
Survival of human adenoviruses, noroviruses, indicator bacteriophages and bacteria in sewage-contaminated streaming waters: Assessing the impact of environmental factors

Wednesday, March 13, 09:00–11:00

Plant pathogens/ Geomicrobiology: From rocks to communities and genomes II

Salon Danzig

Chair: Michael W. Friedrich
Co-Chair: Helge Weingart

PPAV001
09:00
*D. PLETZER, H. WEINGART
RND-type multidrug efflux pumps of the fire blight pathogen *Erwinia amylovora*

PPAV002
09:15
*A. DJAMEI, A. GHOSH, G. BANGE, R. KAHMANN
Elucidating the translocation of the fungal effector Cmu1

PPAV003
09:30
*F. DRECHSLER, H. GHAREEB, J. SCHIRAWSKI
Mapping functional domains of a secreted effector of *Sporisorium reilianum*

PPAV004
09:45
*P. KUSARI, M. LAMSHÖFT, S. KUSARI, M. SPITELLER, O. KAYSER
Profiling of quorum sensing response and biocontrol potential of endophytic bacteria harbored in *Cannabis sativa* L.

GEOV009
10:00
*M. COOPER, F. SONNTAG, A. SONNABEND, D. WONDROUSCH, G. SCHÜÜRMANN L. ADRIAN, A. WAGNER
Electron density modelling for the fate prediction of halogenated heteroaromatics in anaerobic environments

GEOV010
10:15
*D. KANAPARTHI, B. POMMERENKE, M. DUMONT
Chemolithoautotrophic nitrate-dependent Fe(II) oxidizing nature of members of genus *Thiomonas*

GEOV011
10:30
*M. BLASER, R. CONRAD
Carbon isotope fractionation and the acetyl-CoA pathway

GEOV012
10:45
*M. SUHR, S. MATYS, J. RAFF, K. POLLMANN
Metal interaction processes with cell wall components of Gram-positive bacteria studied by QCM-D

From soil to the ocean

Salon Franzius

Chair: Jan Kuever
Co-Chair: Alban Ramette

MDV001
09:00
*A. KLINDWORTH, E. PRUESSE, T. SCHWEER, J. PEPLIES, C. QUAST, M. HORN, F.O. GLÖCKNER
***In silico* evaluation of primer and primer pairs for 16S ribosomal RNA biodiversity**

MDV002
09:15
*S. WALLISCH, M. ENGEL, T. RATTEI, W. HELLER, S. STICH, F. FLEISCHMANN, J.C. MUNCH, M. SCHLOTER
Microbial communities involved in leaf litter degradation of annual and perennial plants

MDV003
09:30
F. WEMHEUER, *B. WEMHEUER, D. KRETZSCHMAR, R. DANIEL, S. VIDAL
Multitrophic interaction between microorganisms, plants and herbivores: Does fertilizing, mowing or herbivory on plants alter the microbial community composition in the rhizosphere?

MDV004
09:45
*O. SCHMIDT, M.A. HORN, S. KOLB, H.L. DRAKE
***Acidobacteria*, *Bacteroidetes* and unclassified Bacteria dominate the anaerobic degradation of cellulose in fen soil**

MDV005
10:00
*C. LÜKE, A. HO, P. FRENZEL
Biogeography of methane oxidizing bacteria: The β-diversity of *pmoA* genotypes in tropical and subtropical rice paddies

MDV006
10:15
*C. KARWAUTZ, M. STÖCKL, T. LUEDERS
Microbial biofilms in a unique methane-fueled iodine-rich cave ecosystem

MDV007
10:30
*I. SÁNCHEZ-ANDREA, R. AMILS, A.J.M. STAMS, J. SANZ
Microbial community in anaerobic sediments of Tinto River determined by culture-dependent and culture-independent methods

MDV008
10:45
*J. SEIFRIED, A. WICHELS, G. GERDTS
Diversity and dynamics of bacterial populations in marine bioaerosols

Dissecting virulence II

Salon London

Chair: Roland Benz
Co-Chair: Bernard A. M. van der Zeijst

MMAV009
09:00
*M. KNOPP, D.I. ANDERSSON
Rapid and efficient compensation of low-fitness mutants resistant to several clinically important antibiotics

MMAV010
09:15
*A. SCHMEISKY, D. REUSS, S. GROSSHENNIG, J. BUSSE, J. STÜLKE
Carbon source dependent hydrogen peroxide production in Mycoplasma species

MMAV011
09:30
M. VAN OOSTEN, T. SCHAEFER, J.A.C. GAZENDAM, K. OHLSEN, E. TSOMPANIDOU, M.C. DE GOFFAU, H.J.M. HARMSEN, L.M.A. CRANE, K.P. FRANCIS, L. CHEUNG, M. OLIVE, V. NTZIACHRISTOS, G.M. VAN DAM, *J.M. VAN DIJL
Real-time *in vivo* imaging of invasive- and biomaterial-associated bacterial infections using fluorescently labeled vancomycin

MMAV012
09:45
*C. ÜNAL, B. SINGH, C. FLEURY, K. SINGH, L. CHÁVEZ DE PAZ, G. SVENSÄTER, K. RIESBECK
Role of QceB/C (FirR/S) in biofilm formation of non-typeable *Haemophilus influenzae* (NTHi)

Wednesday, March 13, 09:00–11:00

MMAV013

10:00

*S. BERESWILL, L.-M. HAAG, A. FISCHER, B. OTTO, R. PLICKERT, A.A. KÜHL, U.B. GÖBEL, *M.M. HEIMESAAT

Campylobacter jejuni induces acute enterocolitis in gnotobiotic IL-10$^{-/-}$ mice via Toll-like-receptor-2 and -4 signaling

MMAV014

10:15

*M. BURGHARTZ, V. HERING, C. LASSEK, M. ROHDE, R. DANIEL, D. JAHN, M. JAHN

Multiresistant uropathogenic _Myroides_ sp. with unusual morphology features

MMAV015

10:30

*M. ADLER, M. ANJUM, D. ANDERSSON, L. SANDEGREN

Role of high-level amplification of β-lactamase genes in carbapenem resistant _E. coli_

MMAV016

10:45

*S.F. HUSSAIN, A. KHATOON, S. HASAN, S.M. SHAHID, M. ISMAIL, A. AZHAR

Discriminative detection of methicillin resistant _Staphylococcus aureus_ carrying Panton-Valantine Leukocidin gene in clinical isolates

ISV01
EHEC-Bakterien – gestern und heute

*H. Karch[1]
[1]*Westfälische Wilhelms-Universität Münster, Zentrum für Klinisch-Theoretische Medizin I, Institut für Hygiene, Münster, Germany*

No abstract submitted.

ISV02
Marine sediment hardwired by microbes

*L.P. Nielsen[1]
[1]*Aarhus University, Department of Bioscience - Microbiology, Aarhus C, Denmark*

No abstract submitted.

ISV03
Microbial uptake of depleted phosphate in the North Atlantic subtropical gyre

*M.V. Zubkov[1]
[1]*National Oceanography Centre, Southampton, Ocean Biogeochemistry & Ecosystems Research Group, Southampton, United Kingdom*

Subtropical gyres cover ~40% of the surface of the Earth, forming vast and expanding oceanic oligotrophic ecosystems. The North Atlantic subtropical gyre is particularly depleted in inorganic phosphate (P_{in}). Within a few hours the entire stock of bioavailable P_{in} in the surface waters of that gyre could be taken up by planktonic microbes, who apparently keep substantial extracellular pools of P_{in}. Among these microbes, the numerically dominant SAR11 alphaproteobacteria effectively compete for P_{in} with *Prochlorococcus* cyanobacteria, despite the latter being the main CO_2-fixers in the gyre. The other main oceanic CO_2-fixers - the smallest algae - play a minor role in overall microbial P_{in} uptake, presumably obtaining phosphorous by preying on bacterioplankton. How this changes our views on microbial acquisition of P_{in} will be discussed.

ISV04
Genomic insights into Phytoplankton capabilities

*V. Armbrust[1]
[1]*University of Washington, School of Oceanography, Dept. of Biological Oceanography, Seattle, WA, United States*

No abstract submitted.

ISV05
Microalgal biotechnology

*R. Wijffels[1]
[1]*Wageningen University, Bioprocess Engineering, Wageningen, Netherlands*

No abstract submitted.

ISV06
A programmable DNA-binding domain

*J. Boch[1]
Martin Luther University Halle-Wittenberg, Institute of Biology, Halle (Saale), Germany

The DNA-binding domain of TALEs (transcription activator-like effectors) from Gram-negative plant-pathogenic *Xanthomonas* bacteria has become an important tool for the programmable and specific targeting of DNA. Natural TALEs function as transcription factors which are injected via a type III secretion system into plant cells to support bacterial colonization of host plants. TALE proteins bind to DNA via near-identical tandem repeats of 34 amino acids. Each repeat recognizes one base in the target DNA sequence via repeat-variable diresidues (RVDs). The simple and modular repeat architecture allows rearrangement of TALE repeats to generate artificial TALEs with virtually any tailored DNA-binding specificity[1]. Highly specific genome-editing TALE nucleases can be engineered for targeted mutagenesis in plants and a wide variety of other eukaryotic organisms. We analyzed specificities and activities of TALEs experimentally in a transient reporter system using *Agrobacterium*-mediated expression *in planta*. Different RVDs exist in nature and I will present results on RVD specificities and efficiencies[2], as well as the design of programmable gene switches and programmable precision mutagenesis tools. Understanding TALE specificity now also allows to predict which plant genes are virulence targets of *Xanthomonas* TALEs. TALEs are versatile virulence factors for the bacterial pathogen and exceptional tools for biotechnology.

1. Boch, J., Scholze, H., Schornack S., Landgraf, L., Hahn, S., Kay, S., Lahaye, T., Nickstadt, A., and Bonas, U. (2009) Breaking the code of DNA binding specificity of TAL-type III effectors. Science **326**, 1509-1512.

2. Streubel, J., Blücher, C., Landgraf, A., and Boch, J. (2012) TAL effector RVD specificities and efficiencies. Nat. Biotechnol. **30**, 593-595.

ISV07
Comparative genomics of fungal plant pathogens and mechanisms of adaptation to their host plants

*P.J.G. de Wit[1]
[1]*Wageningen University, Laboratory of Phytopathology, Wageningen, Netherlands*

We compared the genomes of the fungal plant pathogens *Cladosporium fulvum* and *Dothistroma septosporum* that are phylogenetically closely related, but have different lifestyles and infect different hosts. *C. fulvum* is a biotroph that infects tomato, while *D. septosporum* is a hemibiotroph infecting pine. The genomes of these fungi have a similar set of genes but differ significantly in size (*C. fulvum* >61.1 Mb; *D. septosporum* 31.2 Mb), which is mainly due to the difference in repeat content. Repeat-rich areas in *C. fulvum*, which primarily consist of retrotransposons, were enriched for species-specific genes including those encoding secreted effector proteins. Several previously cloned effector genes from *C. fulvum* are present in *D. septosporum* and some of them (*Ecp2* and *Avr4*) are recognized by tomato Cf resistance proteins and cause a Cf-mediated hypersensitive response. Some gene clusters encoding the dothistromin toxin, well studied in *D. septosporum,* are conserved in *C. fulvum,* although in this fungus some of the genes are pseudogenized or not expressed *in planta*. *C. fulvum* produces the species-specific enzyme α-tomatinase, absent in *D. septosporum,* that detoxifies α-tomatine present in high concentrations in tomato enabling it to colonize tomato. In the two fungi and other *Dothideomycetes* intron-like elements were identified; these are highly structured near-identical introns present in different genes that are multiplied by a yet unknown mechanism. Overall, comparison of the two genomes shows that closely related plant pathogens have adapted to different hosts and lifestyles by different mechanisms including gene innovations, pseudogenization and gene regulation.

ISV08
Microbes inside – Interactions at the intestinal interface

*W. de Vos[1]
[1]*WU Agrotechnologie & Voedingswetenschappen, Laboratory of Microbiology, Wageningen, Netherlands*

No abstract submitted.

ISV09
Individual look at microbes – Single cell genomics defies averages

*R. Stepanauskas[1]
[1]*Bigelow Laboratory for Ocean Sciences, Single Cell Genomics Center, East Boothbay, United States*

The vast majority of microorganisms on Earth resist cultivation, making it impossible to study them by classical microbiology tools. Most culture-independent methods, such as pyrotag sequencing and metagenomics, are effective for individual gene discovery, but they seldom provide information about linkages among genes in biochemical pathways and genomes. As a result, metabolic features of most operational taxonomic units, as defined by their SSU rRNA sequences, remain unknown, while most community DNA, RNA and protein fragments cannot be assigned to a particular microorganism. Single cell genomics is a novel research approach that helps bridging this major knowledge gap, enabling the analysis of entire genomic blueprints of uncultured microorganisms.
In my presentation I will review the latest developments in microbial single cell genomics technology and resulting discoveries. In one example, we found chemoautotrophy pathways in abundant, yet uncultured bacteria inhabiting the vast expanse of the dark ocean, improving our understanding of the global carbon cycle. In another example, we used single cell genomics to decipher in situ interactions of uncultured protists, uncovering their grazing preferences and viral infections. My third example, based on a large-scale single cell genomics study of the predominant bacterial lineages in the

ocean, reveals the prevalence of genome streamlining and latitudinal biogeographic patterns of surface ocean bacterioplankton. Lastly, a single cell genomics study focused on phylogenetic lineages with no cultured representatives shows unexpected metabolic features that extend our understanding of biology and challenge established boundaries between the domains of life. Collectively, these results demonstrate how single cell genomics overcomes some of the major technical challenges that microbiology has been facing so far, enabling greatly improved understanding of the ecology and evolutionary histories of microbial life.

ISV10

Anammox bacteria – microbes with identity issues

*L. van Niftrik[1]
[1]*Radboud University Nijmegen, Dept. of. Microbiology, Institute for Water & Wetland Research, Nijmegen, Netherlands*

Anammox bacteria convert ammonium and nitrite to nitrogen gas to obtain energy for growth. The anammox reaction was deemed impossible until its discovery in the early 1990s. Now, anammox is recognized to contribute significantly to oceanic nitrogen loss and is estimated to be a major source of gaseous nitrogen on Earth. In addition, anammox bacteria are extremely valuable for wastewater treatment where they are applied for the removal of ammonium. Besides their importance in industry and the environment, anammox bacteria defy some basic biological concepts. Whereas most other bacteria have only one cell compartment, the cytoplasm, anammox bacteria have three independent cell compartments, from out- to inside; the paryphoplasm, riboplasm and anammoxosome. The anammoxosome is the largest cell compartment and is proposed to be dedicated to energy transduction. As such it would be analogous to the mitochondria of eukaryotes.
The riboplasm contains the nucleoid and ribosomes and the paryphoplasm has a yet unknown function. Having three cellular compartments poses challenges to protein sorting, substrate transport and cell division and it is largely unknown how anammox bacteria achieve these functions.
In addition, anammox bacteria are proposed to have an atypical cell wall devoid of both peptidoglycan and a typical outer membrane. We use cell fractionation, proteomics and immunolocalization studies combined with advanced (cryo-)electron microscopy techniques (such as electron tomography) to study the ultrastructure and function in anammox bacteria.

ISV11

Systemic analysis of human-associated microbes – Lessons from a tiny bacterium and a large community

*P. Bork[1]
[1]*EMBL Heidelberg, Bioinformatics, Heidelberg, Germany*

Bacteria share many more molecular features with eukaroytic cells than currently appreciated and are convenient models for the study of many fundamental biomolecular processes. I will illustrate the power of such models using one of the smallest bacteria, Mycoplasma pneumoniae. Data on the transcriptome, metabolome and proteome have been consistently generated and integrated to reveal a wealth of information about the biology of a genome-reduced bacterium that was found to be remarkably complex (Kuehner et al, Science 2009, Yus et al., Science 2009, Guell et al., Science 2009, Van Noort et al., Mol.Sys.Biol. 2012). In order to utilize bacteria for human health, yet another layer of complexity has to be understood, that of the interactions of many bacteria forming microbial communities. The recent advent of environmental sequencing (metagenomics) enabled the collection of genomic parts lists of various microbial communities, but our understanding of their functioning still remains limited. Using the human gut as an example, I will briefly introduce into recent technological advances (Qin et al., 2010) and will describe recent findings on the stratification of such communities in the human population and the applications relevant to human health. For example,i) the gut microbial communities of each human individual can be classified into three enterotypes (Arumugam et al., Nature 2011) ii) each human individual appears to carry a unique set of microbial strains leading to individual genomic variation patterns (Schloissnig et al., Nature 2013) and iii) several human diseases show significant associations with microbial markers identifiable in metagenomic data sets.

Kuehner S et al., Science. 2009 Nov 27;326(5957):1235-40.
Yus E et al., Science. 2009 Nov 27;326(5957):1263-8.
Guell M et al., Science. 2009 Nov 27;326(5957):1268-71.
Van Noort V et al., Mol.Sys.Biol. 2012, Feb 28;8:571.
Qin J et al., Nature. 2010 Mar 4;464(7285):59-65.
Arumugam M et al., Nature. 2011 May 12;473(7346):174-80.
Schloissnig S et al., Nature 2013 Jan 3;493(7430):45-50.

ISV12

Network-based data integration for microbial systems biology

*K. Marchal[1,2,3]
[1]*Center of Microbial and Plant Genetics, Leuven, Belgium*
[2]*VIB, Department of Plant Systems Biology, Ghent, Belgium*
[3]*Ghent University, Department of Plant Biotechnology and Bioinformatics, Ghent, Belgium*

With the advent of new molecular profiling techniques, genome-wide datasets that describe interactions between molecular entities (i.e., mRNA, proteins, metabolites,…) are being generated at an ever increasing pace. These datasets each measure a specific type of interaction that is active under certain conditions or that occurs as a response to specific environmental signals. Most of these data, present in the public domain provide a wealth of information that can be used to infer novel biological findings or as a scaffold against which own findings can be interrogated.
In this presentation I will show how publicly available omics data can be used to infer interaction networks and how these networks can subsequently be used to help interpreting the results of own in house generated genomics datasets.

ISV13

The end of the microbial redox tower?

*M. Strous[1]
[1]*Max Planck Institute for Marine Microbiology, Bremen, Germany*

No abstract submitted.

ISV14

Microbial resources and their management – Where are we?

*W. Verstraete[1]
[1]*LabMET, Ghent, Belgium*

No abstract submitted.

ISV15

Ammonia oxidizing Archaea: Physiology & evolution

*C. Schleper[1]
[1]*University of Vienna, Dept. of Genetics in Ecology, Vienna, Austria*

No abstract submitted.

ISV16

Discovery and physiology of "impossible" micro-organisms in Nitrogen and Methane Cycles

*M. Jetten[1]
[1]*Radboud University of Nijmegen, Institute for Water and Wetland Research, Nijmegen, Netherlands*

Anaerobic oxidation of ammonium by anammox bacteria or anaerobic oxidation of methane by M*ethylomirabils oxyfera* bacteria are recent discoveries in the nitrogen and methane cycle catalyzed by completely unrelated microbes. However, the processes share many interesting microbial aspects. Both processes were once deemed to be biochemically impossible and non-existent in nature, but have now been identified as important players in global nitrogen and methane cycling (Strous et al Nature 2006). Their detection and identification was possible by the progress made in molecular ecology, genome sequencing, cellular biology, tracer studies, use of lipid biomarkers, and dedicated reactor systems with efficient biomass retention (Raghoebarsing et al Nature 2006). Therefore the microbes responsible for the processes are now available in enrichment cultures that produce enough cells to investigate their physiology by transcriptome and proteome experiments. These studies showed that anammox bacteria make the rocket fuel hydrazine by novel multiheme complexes (hydrazine synthase and hydrazine dehydrogenase; Kartal et al Nature 2011) that are located in the anammoxosome organelle (van Niftrik and Jetten , 20102). The *M. oxyfera* bacteria turned out to have a new intra-aerobic metabolism.

They are able to produce their own oxygen by conversion of 2 NO into O_2 and N_2 by a putative NO dismutase. On the basis of the nod and hzsA genes new diagnostic PCR primer sets have been developed to detect anammox and M. oxyfera bacteria in oxygen-limited environments.

Strous et al Nature 2006 Deciphering the evolution and metabolism of an anammox bacterium from a community genome. ***Nature*** 440: 790-794
Raghoebarsing et al 2006 A microbial consortium couples anaerobic methane oxidation to denitrification. ***Nature*** 440: 918-921
Ettwig et al 2010 Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. ***Nature*** 464, 543-548.
Kartal et al 2011 Molecular mechanism of anaerobic ammonium oxidation. ***Nature*** 479, 127-130.
Van Niftrik L, and Jetten MSM (2012) Anaerobic Ammonium-Oxidizing Bacteria: Unique Microorganisms with Exceptional Properties. Microbiol. Mol. Biol. Rev., 76(3):585.

ISV17

Microbial ecology of the cocoa bean fermentation process

*L. De Vuyst[1]
[1]*Vrije Universiteit Brussel, Research Group of Industrial Microbiology and Food Biotechnology, Department of Bioengineering Sciences, Brussels, Belgium*

Cocoa bean fermentation is a spontaneous curing process to facilitate pulp removal from as well as drying and color and flavor development of non-germinating cocoa beans. As it is carried out on the farm, cocoa bean fermentation is subject to various operational practices and hence end-products of variable quality are obtained. However, spontaneous cocoa bean fermentations that are carried out with care during four to six days are characterized by a specific succession of microbial activities of three groups of microorganisms, which result in well-fermented fully brown cocoa beans. This has been shown through a multiphasic microbiological approach, involving a culture-dependent analysis, denaturing gradient gel electrophoresis analysis (of PCR amplicons targeting 16S rRNA and 26S rRNA genes of bacteria and yeasts, respectively), metagenomic analysis (16S rRNA gene clone library and 454 pyrosequencing), and meta-metabolomics analysis of samples from spontaneous cocoa bean fermentations carried out in Ghana, Ivory Coast, Brazil, Ecuador, and/or Malaysia. First, yeasts, in particular *Hanseniaspora opuntiae/uvarum* and *Saccharomyces cerevisiae*, are responsible for a fast and consistent anaerobic fermentation of carbohydrates (glucose) into ethanol together with pulp removal through pectinolysis. Second, lactic acid bacteria (LAB), in particular *Lactobacillus fermentum*, are indispensable for citric acid conversion, fructose fermentation and reduction and glucose fermentation to produce lactic acid, acetic acid, and mannitol. Third, acetic acid bacteria, in particular *Acetobacter pasteurianus*, are necessary to oxidize ethanol and lactic acid to acetic acid. Further, additional citric acid convertors (e.g., *Leuconostoc paramesenteroides*) and fructophilic LAB species (e.g., *Fructobacillus pseudoficulneus*) may be found at the start of the fermentation process. Also, it turned out that *Enterobacteriaceae* may participate in the beginning of the fermentation process, thereby contributing to pectinolysis, assimilating citric acid, and producing gluconic acid. Alternatively, gluconic acid production out of residual glucose late into the fermentation process could be ascribed to *Glucon(aceto)bacter*. In addition, the detection of bacteriophage-related sequences reflected *Lactobacillus* as the dominant host. Finally, different new species were discovered, such as *Lactobacillus cacaonum*, *Lactobacillus fabifermentans*, *Weissella fabaria*, *Weissella ghanensis*, *Acetobacter fabarum*, and *Acetobacter ghanensis*. After having tested several of the prevailing microorganisms in appropriate cocoa pulp simulation media to unravel their functional roles and interactions as well as in small plastic vessels containing fresh cocoa pulp-bean mass to evaluate their capacity to dominate the cocoa bean fermentation process, a starter culture composed of appropriate strains of *S. cerevisiae*, *Lb. fermentum*, and *A. pasteurianus* could be formulated to carry out four-day cocoa bean fermentation processes successfully. This starter culture was implemented on several farms in two different cocoa-producing regions (West Africa and Southeast Asia) and their prevalence during fermentation resulted in uniformly fermented dry cocoa beans that gave concomitant milk and dark chocolates with a reliable flavour, independent of cocoa-producing region or fermentation method.

ISV18

Emerging spores – the good, the bad, the ugly

*M. Ehling-Schulz[1]
[1]*University of Veterinary Medicine, Department of Pathobiology, Institute of Functional Microbiology, Vienna, Austria*

The genus *Bacillus*, which comprises the largest group of endospore forming bacteria, confronts food industry with various challenges. Due to their ubiquitous nature, Bacillus spores can enter the food production at several stages resulting in significant economic losses and posing a potential risk to consumers. Numerous *Bacillus* species can cause food spoilage whilst others are well known for their toxigenic potential. However, it is increasingly becoming evident that certain characteristics are rather strain dependent than species specific. For instance, the toxigenic potential of *Bacillus cereus* ranges from low or none in strains used as probiotics and plant growth promoters to extremely high in strains responsible for fatalities. The challenge for current and future *Bacillus* diagnostics is to specifically detect, track and trace potential hazardous strains and, last but not least, to discriminate hazardous strains from non-toxic or even beneficial strains. It is expected that the currently taxonomic focused diagnostics will gradually be replaced by more risk orientated diagnostics.
The most prominent member of the genus Bacillus is the *B. cereus* group, also called *B. cereus sensu lato*. *B. cereus* s.l. comprises several genetically closely related organisms (*B. anthracis, B. cereus sensu stricto, B. thuringiensis, B. mycoides, B. weihenstephanensis* and *B. cytotoxicus*) that show distinct pathogenic characteristics. Because of their medical, food safety and food quality relevance as well as economic importance, several methods for typing and characterization of this interesting group of spore formers have been developed, which provide an excellent 'tool box' for food and clinical diagnostics. In addition, comprehensive Bacillus genome sequencing projects are underway, opening new avenues for 'next generation' diagnostics.

AMV001

Antibiotic resistances panel of 336 *Listeria monocytogenes* strains isolated from German food and patient samples

*M. Noll[1,2], S. Al Dahouk[2]
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The objective of this study was to evaluate the susceptibility of 336 *Listeria monocytogenes* strains isolated from German food and food-processing environments and patient samples to 15 antibiotics currently used in veterinary and human therapy. Susceptibility tests were performed by using an automated 96-well based micro dilution system (Micronaut-S). Ampicillin, amoxicillin, cefotaxim, ceftriaxon, ciprofloxacin, clarithromycin, erythromcyin, gentamicin, imipenem, linezolid, penicillin, rifampin, tetracycline, trimethoprim & sulfamethoxazole and vanacomycin were tested in more than four different concentrations. *Listeria monocytogenes* strains were subdivided to the serotypes 1/2a (n=158), 1/2b (n=32), 1/2c (n=32), 3a (n=2), 3b (n=4), 3c (n=1), 4a (n=2), 4b (n=86), 4ab (n=5), 4c (n=3), 4d (n=3), 4e (n=1), 6a (n=2), 6b (n=4). Among the 336 tested, *L. monocytogenes* of the serotypes 4b were more resistant to antibiotics than other tested serotypes. Strains were mainly resistant to Cefotaxim, Erythromycin, Imipenem and Rifampicin but not to Vanacomycin. The antibiotic resistance panel and the occurrence of multi resistance of *L. monocytogenes* strains were more abundant in this study compared to similar strain panels from other European countries and USA. Enhanced resistance patterns of *L. monocytogenes* might be due to the widespread use of antibiotics and due to the frequent application of surface disinfectants in food production plants and in hospitals.

AMV002

Tn6188 – a novel transposon in *Listeria monocytogenes* conferring tolerance to benzalkonium chloride

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Controlling the food-borne pathogen *Listeria* (*L.*) *monocytogenes* is of great concern for food safety and thus for human health. This is exemplified by recent large listeriosis outbreaks in the USA and Europe. Particularly resistance to quaternary ammonium compounds such as benzalkonium chloride has been observed for many *L. monocytogenes* strains. However, the molecular determinants and mechanisms of benzalkonium chloride resistance of *L. monocytogenes* are still largely unknown. Here we describe Tn6188 a novel transposon in *L. monocytogenes* conferring tolerance to benzalkonium chloride. Tn6188 is related to Tn554 from *Staphylococcus aureus* and other Tn554-like transposons such as Tn558, Tn559 and Tn5406 found in various *Firmicutes*. Tn6188 comprises 5117 bp is integrated chromosomally and consists of three consecutive transposase genes (*tnpABC*), a small multidrug resistance protein family (SMR) transporter for the export of benzalkonium chloride showing high amino acid identity to

Smr/QacC from *S. aureus* and to EmrE from *E. coli*; and a transcriptional regulator. We screened 98 *L. monocytogenes* strains for the presence of Tn6188 using PCR and found Tn6188 in 11 of the analyzed strains - isolates from food and food processing environments of mainly serovar 1/2a. We could show that the susceptibility to benzalkonium chloride of *L. monocytogenes* strains harboring Tn6188 is significantly lower than those of strains without Tn6188.

AMV003

Matrix Lysis – a sample preparation method for recovery of bacterial targets based on solubilization of the sample matrix

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Question: Due to the implementation of critical pathogen levels, direct quantification of food-borne pathogens from food is going to become standard in food risk analysis. Until now major challenges for molecular biological detection and quantification (such as qPCR) of food-borne pathogens are heterogeneous food matrices and large sample quantities. Therefore a major research topic is the development of sample treatment methods prior to subsequent molecular detection and quantification methods, which allow the separation, concentration and purification of the target organisms from the sample matrix. This study describes the modular sample preparation method "Matrix-Lysis" for the quantification of Gram-positive and Gram-negative bacteria from food [1-3].
Methods/Results: Molecular biological (qPCR) and microbiological methods (Plate Count Method) are used to quantify different Gram-positive and Gram-negative bacteria from various artificially and naturally contaminated foodstuffs (milk, cheeses, meat and fish) after Matrix-Lysis.
Artificial contamination experiments show that all bacteria were efficiently recovered from 6.25g - 12.5g food to allow for accurate quantification with detection limits of 10 CFU/g. Matrix-Lysis was also validated Examination of naturally samples resulted in 100% relative accuracy, 100% relative specificity and 100% relative sensitivity compared to the ISO 11290-1 standard method.
Conclusions: Matrix-Lysis is a rapid sample preparation method (< 3h) that is based on the chemical lysis of the foodstuff matrix and subsequent physical separation of the target organisms. The Matrix-Lysis system is designed as a modular system whereby the application flow stays the same for different food classes and pathogens through the use of specialized lysis buffer systems.
The results presented show that Matrix-Lysis allows for reliable quantification of as little as 10 CFU/g foodstuffs, using both molecular biological as well as microbiological methods.

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AMV004

Rapid quantification and classification of bacteria and methanogens by digital image analysis

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The main idea of this study has been to pursue the development of a microscopic analysis system for quantification and classification of environmental cells in a rapid and reliable way. This technology belongs to the cultivation-independent methods to obtain absolute and direct quantification of environmental samples in number per volume (ml). It could be realized by a digital image analysis software, a motorized microscope, and optimized sample preparation technologies. Appropriate sample preparation was a prerequisite for this method such as homogenous distribution of cells on the microscopic slide in a mono layer, adherence of cells and quantification of the sample area on the slide. The optimization of image analysis excluded abiotic particles and differentiated between bacterial cells and untargeted particles like plant debris. Methyl cellulose was used as coating agent for the microscopic slide. Visualization of cells and methanogens could be realized by a stain of SYBR Green I for total cell counting and by auto-fluorescence based on the coenzyme of F_{420} for the analysis of methanogens. The following analysis was performed by image analysis software of Image Pro 7. Validation of this image analysis was carried out by manual counting with a Neubauer Counting chamber. For this, artificial fluorescent nano-particles, monocultures like *Escherichia coli* and *Staphylococcus xylosus* were used for target objects as well as grinded maize and nano-particles for interferring particles. The results showed a good congruence between image analysis and Neubauer counting chamber, representing deviations of 0.2 - 2.6% depending on the cell type. Furthermore, environmental samples from agricultural large scale biogas plants fed e.g.by maize silage and cow manure were analyzed. The number of total cells and methanogens were $3.82*10^{10}$ and $4.02*10^{8}$ with a standard deviation of 6.3% and 4.7% respectively.

AMV005

A proteomic view at the biochemistry of syntrophic butyrate oxidation in *Syntrophomonas wolfei*

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In syntrophic conversion of butyrate to methane and CO_2, butyrate is oxidized to acetate by secondary fermenting bacteria such as *Syntrophomonas wolfei* in close cooperation with methanogenic partner organisms, e.g., *Methanospirillum hungatei*. This process involves an energetically unfavourable shift of electrons from the level of butyryl-CoA oxidation to the substantially lower redox potential of proton and/or CO_2 reduction, in order to transfer these electrons to the methanogenic partner *via* hydrogen and/or formate.
In the present study, all prominent membrane-bound and soluble proteins expressed in *S. wolfei* specifically during syntrophic growth with butyrate, in comparison to pure-culture growth with crotonate, were examined by one- and two-dimensional gel electrophoresis, and identified by peptide fingerprinting-mass spectrometry. A membrane-bound, externally oriented, quinone-linked formate dehydrogenase complex was expressed at high level specifically during syntrophic butyrate oxidation, comprising a selenocystein-linked catalytic subunit with a membrane-translocation pathway signal (TAT), a membrane-bound iron-sulfur subunit, and a membrane-bound cytochrome. Soluble hydrogenases were expressed at high levels specifically during growth with crotonate. The results were confirmed by native protein gel electrophoresis, by formate dehydrogenase and hydrogenase-activity staining, and by analysis of formate dehydrogenase and hydrogenase activities in intact cells and cell extracts. Furthermore, a membrane-bound, internally oriented iron-sulfur oxidoreductase (DUF224) together with soluble electron-transfer flavoproteins (EtfAB) expressed constitutively at high levels was confirmed, as well as the expression of two previously identified butyryl-CoA dehydrogenases.
The findings allow depicting an electron flow scheme for syntrophic butyrate oxidation in *S. wolfei*. Electrons derived from butyryl-CoA are transferred through a membrane-bound EtfAB:quinone oxidoreductase (DUF224) to a menaquinone cycle and further *via* a *b*-type cytochrome to an externally oriented formate dehydrogenase.

AMV006

Proof of principle for a microbial fuel cell biosensor based on *Shewanella oneidensis* outer membrane protein complexes

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Shewanella oneidensis is known for its ability to respire on extracellular electron acceptors. The spectrum of these acceptors includes anode surfaces in a microbial fuel cell. Based on this activity, a versatile *S. oneidensis* based biosensor strain was constructed in which electricity production can be modulated. Construction started with the identification of a usable rate-limiting step of electron transfer to an anode. Thereafter, the sensor strain was genetically engineered to produce a protein complex consisting of the three proteins MtrA, MtrB and MtrF. This complex is associated to the outer membrane and most probably enables membrane spanning electron transfer. MtrF is an outer membrane cytochrome that catalyzes electron transfer reactions on the cell surface. Under anoxic conditions, wild type cells do not express MtrF but rather MtrC as electron transferring outer membrane cytochrome. Still, our analysis revealed that MtrF compared to MtrC overexpression is less toxic to the cell which gives MtrF a superior position for biosensor based applications. Transcription of *mtrA*, *mtrB* and *mtrF* was linked up to an inducible promoter system, which positively reacts to rising L-arabinose concentrations. Current production mediated by this strain was linearly dependent on the arabinose content of the medium. This linear

dependency was detectable over a wide range of arabinose concentrations. The L-arabinose biosensor presented in this study proofs the principle of an outer membrane complex based sensing method which could be easily modified to different specificities by a simple change of the regulatory elements [1].

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AMV007

Production of lipids from lignocellulose with recombinant *R. opacus* PD630 strains

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The microbial conversion of lignocellulosic biomass into second-generation biofuels in an economical competitive process is regarded as key challenge for sustainable energy production. Today's biofuels are mainly derived from grain-based feedstocks and plant biomass whose production is associated with economical, ecological and social problems. While most efforts toward economical production of biofuels in the past aimed mostly on the improvement of ethanol yields, the microbial production of biofuels with properties comparable to petrochemical fuels has attracted more and more interest over the last years. *Rhodococcus opacus* strain PD630 is the model oleaginous prokaryote regarding accumulation and biosynthesis of lipids, which serve as carbon and energy storage and can account up to 87 % of the cell dry mass in this strain (Alvarez *et al*., 1996). It has been considered as production strain for high-value triacylglycerols (TAGs) for the production of biodiesel, monoalkyl esters of short chain alcohols and long chain fatty acids. Its substrate utilization range includes a broad spectrum of different sugars, however, it cannot metabolize the dimeric sugar cellobiose which is the main product of most cellulases and it can also not degrade cellulose. We here report on genetically engineered *R. opacus* PD630 strains that heterologously express 6 different cellulases genes from *Cellulomonas fimi* ATCC484 (*cenABC*, *cex cbhA*) and *Thermobifida fusca* DSM43792 (*cel6A*) which enable *R. opacus* PD630 to degrade cellulosic substrates to cellobiose. The additional expression of the *bglABC* operon from *T. fusca*, encoding two sugar transport proteins and a cytoplasmic β-glucosidase, then conferred the ability to utilize the formed cellobiose for growth and lipid production to *R. opacus*.

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AMV008

Novel high-pressure membrane-capsule bioreactor for the enrichment of anaerobic methanotrophs

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To evaluate the effect of an elevated methane pressure on the anaerobic oxidation of methane (AOM), two membrane-capsule bioreactors were constructed and inoculated with either Eckernförde bay sediment or mixed granular sludge from a methanogenic and a sulphate-reducing UASB reactor. The reactors were kept at an overpressure of 10.1 MPa methane for 240 days. For the Eckernförde bay sediment, AOM rates reached 0.024 mmol g_{vss}^{-1} day^{-1} during the period of incubation. The mixed granular sludge had no AOM activity. Samples from the bioreactor with AOM activity were analyzed using DGGE and clone library construction. By combining these techniques, we were able to pinpoint which archaeal groups were growing in the reactor. Typically, there was proliferation of the ANME2a/2b and ANME-2c archaea involved in AOM. The ANME-1 subtype did not grow, but was present throughout the incubation period. A previous ambient pressure reactor was inoculated with the same sediment and only showed presence of the ANME2a subtype. This indicates a difference in physiology between the different ANME subtypes. DGGE profiling of the bacterial community showed a much less pronounced community shift throughout the incubation period compared to the archaeal community. This needs to be elucidated further and may give more insights into the diversity and interaction of sulphate-reducing bacteria with associated ANME archaea.

AMP001

A regression analysis to identify possible factors that may contribute to sub-optimal bovine spongiform encephalopathy rapid test sample quality in a beef slaughterhouse

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Introduction: Obtaining the appropriate sample is critical to ensure BSE test result integrity. For BSE, this is the brain stem at the level of the obex - the area where abnormal Prion Protein (PrP) is most consistently deposited and fortuitously first detectable. Occasionally suboptimal samples (SO) occur where the obex is absent or unidentifiable, in which case negative results are questionable.
Purpose: The relationship between SO occurrence and the candidate factors of animal age, breed category (dairy, beef breeds native to the British Isles and Continental beef breeds), gender, dehiding method (upward or downward) and sampler identity (n=13) was investigated.
Methods: A stepwise logistic regression model was applied to a dataset containing records of 23,646 animals sampled at the abattoir over a 2 year period from 09.June.2009 to 30.June.2011. Details relating to SO occurence were obtained from reports submitted to the abattoir with each days rapid test results. Details relating to the animals age and date of slaughter, gender and breed were obtained from the Animal Identification and Movement System - the national database held by the Department of Agriculture, Food and the Marine. The abattoir changed its dehiding method on 27.10.2009.
Results: The SO incidence was 0.26%. Results indicate that samplers S_{ahlmk} (OR=5.9; 95%CI=1.9-18.4), S_{dlrkl} (OR=3.5; 95%CI=1.2-10.5), S_{emada} (OR=5.3; 95%CI=2.0- 13.7), bulls (OR=2.7; 95%CI=1.4-5.3), native beef breeds (OR=2.3; 95%CI=1.2-4.5) and continental beef breeds (OR=2.4; 95%CI=1.3- 4.3) had a significant positive effect on SO occurrence. Age and dehiding were found not to have any effect on SO outcome.
Significance: The results inform a basis for risk ranking animals according to breed and gender prior to sampling. The results also highlight the importance of sampler training and motivation. As animal age is not a significant contributing factor samplers are encouraged to perfect their technique by sampling animals younger than the statutory prescribed age (currently 72 months) prior to taking official samples from older animals.
(OR = Odds Ratio).

AMP002

A comparative microbiological analysis of warm versus cold packing of beef tripe

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Introduction: Regulation (EC) 854/2004 stipulates that food business operators must ensure that offal is maintained at <3°C during processing. However, provision is made for meat to be cut at >3°C when the cutting room and slaughterhouse share the same site. Immediately post cutting and packaging, the meat must be chilled to <3°C.
Purpose: This study compares the microbiological quality of beef tripe chilled to less that 3°C prior to packing to that chilled to 3°C after packing.
Methods: For both sets of conditions 50 x 25g samples of beef tripe were taken 24 hours after packaging. Total Viable Counts (TVC) and Enterobacteriaceae counts (TEC) were enumerated using standard methods.
Result: The $LogA_{TVC}$ [$= x + ½(\ln 10.\sigma^2)$] for cold and warm tripe were 4.88 $log_{10}CFUg^{-1}$ and 4.04 $log_{10}CFUg^{-1}$ respectively. These values were found to differ significantly ($p < 0.05$). $LogN_{TEC}$ (calculated by summing the counts in each set and obtaining the log of the sum) and $\%Neg_{TEC}$ (those samples with counts less than the detection limit) for cold tripe were $2.48g^{-1}$ and 82% respectively whilst those for warm tripe were $2.90g^{-1}$and 70% respectively.
Significance: The results indicate the superior microbial quality of cold tripe with implications for its shelf-life and safety.
Though legislation has provided for warm tripe production, chilling prior to packing must be considered best practice.

AMP003

Assessment of microbiological safety of spices from Serbian market in year 2012

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Modern food production requires a variety of tastes, and this is achieved by adding various combinations of spices in the production process so quality of the spices can greatly affect quality of the finished product. Spices are often contaminated with various microorganisms and their spores that can cause deterioration of food.
According to mentioned the goal of this study was to evaluate microbiological safety of spices (pepper, ground red pepper, cumin, cinnamon, laurel, basil, garlic etc.) from Serbian market. Total number of analyzed samples was 500. Samples of spices were collected in various retail shops and factories during the year 2012 and analyzed on total number of microorganisms, *Escherichia coli*, *Bacillus cereus*, molds and yeasts according to Guidelines for the application of microbiological criteria for foods (Republic of Serbia, Ministry of agriculture, forestry and water management, first edition, June 2011). All microorganisms were tested according to ISO methodology: total number of microorganisms SRPS EN ISO 4833:2008, E. coli SRPS ISO 16649-2:2008, B. cereus SRPS EN ISO 7932:2009, molds and yeasts SRPS ISO 21527-2:2011. Analysis of total number of microorganisms showed that 58.0% of analyzed samples were satisfactory, 40.2% acceptable and 1.8% unsatisfactory. Concerning the analysis of yeasts and molds 62.4% of analyzed samples were satisfactory, 34.6% acceptable and 3.0% unsatisfactory. As far as *E. coli* and *B. cereus* are concerned 93.0% and 91.2% of analyzed samples were satisfactory, 5.8% and 7.4% acceptable, and 1.2% and 1.4% unsatisfactory, respectively. However 87% of all unsatisfactory results comes from the analysis of ground red pepper.
Based on obtained results it can be concluded that ground red pepper is the most contaminated spice. However this spice, on Serbian territory, is mostly used in products that are being further heat treated therefore it cannot endanger the safety of users of the finished product.

AMP004

Screening of materials for electrodes in a microbial fuel cell

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The literature is rich in reports about the performance of microbial fuel cells (MFC). Nevertheless, there is no general procedure for a characterization regarding MFCs with different anode materials and exoelectrogenic microorganisms. We present here an extensive characterization of different anode materials using *Geobacter* and *Shewanella* species as exoelectrogens and suggest a number of values that should be recorded for a sufficient characterization.
First, we claim the necessity to measure the time that is needed in a galvanostatic step to reach a steady potential, which is characterized by the biochemical abilities of the microbes as well as the ability of the material to serve as a biofilm carrier. We observed that different carbon based anode materials show drastic variations for the time needed to reach a steady potential and that this value is also dependent on the microorganisms used. Thereafter, we propose a current sweep as best method to establish to what extend the physiology of exoelectrogens can support the electron uptake by the potentiostat. The slope of the potential over current graph as well as the current at which the potentiostat starts to enable water hydrolysis to support the current (now referred to as *limiting current density* (LCD)) should be recorded as further necessary data for MFC characterization. Again these values depend on the anode material but also on the microorganisms. All data should at least be normalized to the size of the anode. A combination of these measurements with fluorescent *in situ* hybridization (FISH) allows a correlation of the limiting current density to anode coverage by the microbes. Further correlation to the whole cell number (planktonic and sessile) using quantitative PCR allows to differentiate the role of planktonic cells in MFC performance.

AMP005

Propionic acid degradation in biogas plants

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Anaerobic digesters producing natural biogas may suffer from propionic acid accumulation as a result of process instabilities. Due to its slow degradation performance and its microbial toxicity, propionic acid hampers process stabilizing measures. In order to fight propionic acid accumulation in biogas plants, this study attempts to identify and isolate its degrading microbes.
Incubation of serial diluted reactor samples led to a stabile propionic acid degrading syntrophic consortium, which was deployed for subsequent isolation and identification procedures. Frequent HPLC measurements showed its stable degradation. Fluorescence microscopy and amplification of archaeal 16 S rDNA revealed that *Methanoculleus* sp. was the methanogenic partner in the syntrophic consortium. Consequently, *Methanoculleus bourgensis* 3045^T was used as hydrogen scavenging co-microbe. Serial dilutions with *Methanoculleus bourgensis* 3045^T were conducted to isolate propionic acid degrading bacteria from the consortium. Furthermore, micromanipulation techniques were applied to separate single cells out of the consortium.
In future, pure cultures of propionic acid degraders may serve as a tool for reconditioning propionic acid polluted digesters. In addition, the species information contribute to the establishment of a core-microbiom for biogas plants.

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AMP006

Investigating the heat resistance mechanisms of the infant pathogen *Cronobacter sakazakii*

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Cronobacter sp., formerly classified as *Enterobacter sakazakii*, has been implicated in several incidents as the cause of meningitis and enterocolitis with high mortality rates in premature infants resulting from feeding with contaminated powdered infant formula (PIF) [1].
Cronobacter bacteria usually enter the PIF production process as long-term stationary phase cells (pre-dried during starch preparation), which seems to enhance their heat resistance by several magnitudes compared to exponentially growing bacteria. Since pasteurization efficiency is crucial in PIF production, we are investigating the mechanisms by which this bacterium seems to even survive temperatures up to 80°C at least in a VBNC state.

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AMP007

Proof of concept for recombinant cellular controls in quantitative molecular pathogen detection

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Question: Real-time PCR for food pathogen detection is mostly used with internal amplification controls to monitor the enzymatic reaction and for determination of the efficiency of the reaction. Preliminary methodical steps such as sample preparation and DNA isolation and purification are not included in this kind of control and if at all, checked by external controls. This does not allow for control of single samples and thus negative results imply the possibility of false verification of the pathogen status of the investigated food samples. For this purpose there is need for an internal process control covering the whole detection process. The aim of this study was to develop and characterize an internal process control based on a model organism as close related to the actual target pathogen as possible which does not influence the quantitative results of the underlying method for detection of the aimed pathogen.

Methods: A Δ-*prfA L. monocytogenes* EGDe strain was cloned with a phage insertion vector to result in a single copy inserted artificial real-time PCR target (IAC) amplified with the primers binding the *prfA* locus of *L. monocytogenes* resulting in a fluorescence signal not interfering with the respective signal of the *L. monocytogenes* wild type strain.
Results: The *Δ-prfA L. monocytogenes* EGDe strain was characterized *as L. monocytogenes EGDe*, the single copy status of the DNA insertion was demonstrated and the strain was used in context with matrix lysis sample preparation both with artificially and naturally contaminated food samples to demonstrate the use of the control. The resulting corrected values as obtained by the whole molecular detection protocol corresponded to the respective values of contamination as determined according to ISO 11920-2.
Conclusions: The internal sample process control based on IAC+, Δ-*prfA L. monocytogenes* EGDe enables in-sample control for real-time PCR detection of *L. monocytogenes* in food samples. The application of internal sample process controls is essential to cover all steps and integrated methods which are included in molecular biological pathogen detection to provide reliable results.

AMP008

Performance testing of selective enrichment media for *L. monocytogenes* using single bacterial cell manipulation

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Question: In the present study, a recently developed method for single bacterial cell manipulation (SBCM) was applied to validation of enrichment media. The purpose of this study was performance testing of selective and unselective enrichment media in the range of < 10 cells avoiding stochastic effects caused by dilution.
Methods: The performance of Oxoid One broth Listeria and Half-Fraser broth was compared to *tryptone soy* broth with 6% yeast extract (TSB-Y). The growth of both stressed and unstressed cells was investigated. *L. monocytogenes* cells were manipulated by SBCM as previously published. Inocula of 1, 2, 3 and >3 cells were added to the respective media and the samples were analyzed after 24 and 48 hours of incubation by determination of the optical density of the offspring cultures. Additionally enrichment broths/samples were streaked on selective and unselective solid media by the semi-quantitative three-loop technique and real-time PCR targeting the prfA gene were performed for determination of cell counts of the offspring cultures.
Results: A significant difference in the performance of selective media compared to unselective TSB-Y was identified for unstressed *L. monocytogenes* cells. Chilling stress resulted in no offspring cultures of *L. monocytogenes* in Half-Fraser broth as well as in Oxoid One broth Listeria when 1 cell was inoculated compared to 70% offspring in TSB-Y. A coherence of increasing cell numbers in the inoculums to the number of positive offspring cultures was determined.
Conclusions: The use of SBCM generating single cell inocula independent from stochastic effects caused by dilution is a novel and successful tool in performance testing of enrichment media supporting conventional validation procedures. This approach avoids the influence of Poisson distribution and enables the direct evaluation of bacterial cell growth in the range of < 10 initial cells. This provides additional information for more precise determination of the performance of selective and unselective media.

AMP009

Population dynamics in wheat stillage based biogas reactors

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Bioethanol production from wheat stillage yields waste water suitable for biogas production. After the conversion of wheat to ethanol and the distillation process high amounts of lignocellulose and dead yeast cells remain untreated (stillage). Using an inoculum from a high-temperature biogas plant a microbial community was enriched able to convert wheat stillage to biogas at 55°C.
To analyze the microbial community in the model biogas reactor sequence analysis of 16S rRNA gene-tags was conducted resulting in 23,000 bacterial and archaeal gene sequences. The studies indicate the predominance of Archaea of the genera *Methanosaeta* and *Methanothermobacter*, while *Thermotogae, Elusimicrobia*, *Chloroflexi*, *Firmicutes*, and *Synergistes* were the predominat bacterial phyla.
The archaeon *Methanothermobacter thermautotrophicus* and the bacteria *Defluviitalea saccharophila*, *Lutispora thermophila, Thermoanaerobacter thermosaccharolyticum, Clostridium succinogenes, Thermohydrogenium kirishiense* and *Lachnospira* sp. were isolated by anaerobic serial dilution techniques. Enrichment cultures using acetate as sole carbon source and various antibiotics resulted in the selective growth of an unknown axenic culture of *Methanosaeta*. Characterization of growth conditions highlight relevant differences to the type strain *Methanosaeta thermophila* being its closest relative.
To investigate dynamics and stability of the microbial community during process modifications such as increase in loading rate and addition of accumulating intermediates, denaturing gradient gel electrophoresis (DGGE) and fluorescence *in-situ* hybridization (FISH) was performed. The studies revealed the presence of a robust consortium of methanogenic Archaea with variability regarding *Methanosaeta* and *Methanosarcina*, in particular. The results will be compared to studies from a pilot plant based on the same substrate.

AMP010

Field trials to test the application of a bacteriophage-cocktail in broiler farms to reduce *Campylobacter* spp

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At slaughterhouse level approximately 80 % of poultry carcasses are contaminated with *Campylobacter* spp.. This contamination with the most common pathogen causing foodborne human enteritis is mainly due to contamination of the slaughter line with gut content. The reduction during primary production is considered as the most effective strategy to reduce the number of cases of human campylobacteriosis by the European Food Safety Authority (EFSA). Currently no reliable method to reduce *Campylobacter* spp. in broiler flocks is available. A significant reduction of intestinal colonization of *Campylobacter* in broilers could be achieved by the use of bacteriophages. Field studies were carried out to show whether the results found under laboratory conditions with a well characterized *Campylobacter* isolate can be reproduced in commercial broiler flocks. A cocktail containing four *Campylobacter*-specific bacteriophages that was previously tested *in vitro* and *in vivo* was applied in three *Campylobacter*-positive broiler farms. One house was selected as the trial house, the other one as the control. Fecal samples were taken in both houses before the bacteriophage application in the trial house and were quantitatively analyzed for *Campylobacter* and bacteriophages. The bacteriophage cocktail was applied via drinking water. The applied dose of $\log_{10}$ 7.5 pfu/animal was confirmed in two of the trials by drinking water analysis, while in the third trial a dose of $\log_{10}$ 5.8 pfu/animalwas found. The progression of the intestinal *Campylobacter* colonization in the trials groups clearly differed from those of the control groups. In one of the trials a significant reduction of $\log_{10}$ 3.2 cfu/g caecum content could be demonstrated at the slaughterhouse (P= 0.0011). One day after the phage application no *Campylobacter* could be detected anymore (detection limit 50 KbE/g, P= 0.0140).

AMP012

Antifungal activity in seed coat extracts of *Theobroma cacao* L.

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Seed coat is an important tissue for the regulation of imbibition and maintenance of the integrity of seed, and it is also the first seed barrier encountered by pests and pathogens. Seed cotyledons contain an array of proteins that may be involved in the protection of quiescent seeds against

fungi. Now we know that seed coat from *Theobroma cacao L.* seeds contains an antifungal activity. In the present study inhibition tests of seed coat extracts against microorganisms isolated from cocoa bean fermentations were performed. Seed coat was extracted using surface-sterilization and filter-sterilization. To determine antifungal activity in seed coat extract agar diffusion and broth microdilution tests were used. Seed coat extract can inhibit growth of fungi (e.g. *Aspergillus niger, Aspergillus flavus, Aspergillus parasiticus, Mortierella isabellina, Penicillium citrinum, Penicillium purpurogenum, Penicillium roquefortii*). Yeast (e.g. *Candida krusei, Candida lipolytica, Candida guilliermondii, Cryptococcus laurentii, Rhodotorula mucilaginosa, Rhodotorula rubra, Saccharomyces cerevisiae, Schizosaccharomyces pombe*). No inhibition effect could be detected against gram positive bacteria (e.g. *Bacillus subtilis, Staphylococcus aureus, Lactobacillus plantarum, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus reuteri, Propionibacterium freudenreichii*) and gram negative bacteria (e.g. *Escherichia coli, Acetobacter pasteurianus, Acetobacter tropicalis, Acetobacter pomorum, Gluconobacter frateurii, Acetobacter orientalis*). The minimum inhibitory concentration (MIC) of seed coat extract was determined. 25 mg/mL of seed coat extract can inhibit growth of fungi (e.g. *Penicillium citrinum, Aspergillus niger, Penicillium purpurogenum*), 10 mg/mL seed coad extract can inhibit growth of yeasts (e.g. *Saccharomyces cerevisiae, Rhodotorula rubra, Candida lipolytica*).

AMP013

Low growth temperatures elevate cell yield for mesophilic isolates from food

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In the food and nutrition industry cooling is one of the effective instruments of food preservation and food protection. While the decrease of growth rates at low temperatures is a well-known effect only few information are available about the impact of low incubation temperatures on cell yield.
We isolated several mesophilic strains from food samples which showed a significantly higher cell yield after growth at 10°C than after growth at 30°C. As expected, all of these isolates showed lower growth rates at 10°C than at 30°C. This effect was shown for isolates of the genera *Bacillus*, *Paenibacillus*, *Escherichia*, *Pedobacter* and *Listeria*. Type strains of the respective genera were obtained from culture collections and were analyzed for growth yield at low temperature under identical conditions. In contrast to the isolates none of the type strains showed a similar negative correlation between growth temperature and cell yield.
Our data reveal that low incubation temperature can have a positive effect on cell yield. This effect was shown for isolates from food samples but not for reference strains held at culture collections. Because cell number in chilled food has major impacts on food quality, food spoilage and risk potential for consumer, our results has an impact on cold chain management and the assessment of food spoilers under low-temperature conditions.

AMP014

Sanitation of liquid manure by a full-scale thermophilic biogas plant at high loading rate

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The aim of this study was to examine the effectiveness of a successful sanitation of digestate by thermophilic anaerobic fermentation (temperature >50°C). Digestate is the liquid or solid residue from the fermentation of biomass in a biogas plant. It is mostly used as an agricultural fertilizer, because of its high nutrient content. According to the new German legal basis (Erneuerbare-Energien-Gesetz: EEG 2012) the use of corn or other renewable resource plants for methane production should be reduced (up to 60% (w/w) mono-substrate). Thus, the admixing with liquid manure becomes more attractive with energy recovery and additional compensation from the EEG. The use of untreated manure as organic fertilizer on agricultural land leads to a closed nutrient cycle. However, it can possibly result in a cycle of pathogens. The spread of pathogens (e.g. EHEC) can be prevented by operating biogas plants under thermophilic conditions. Furthermore, it would leave open the possibility to bring out the digestate in water protection areas. Preliminary results of the investigation of a thermophilic biogas plant with over 70% (w/w) cattle manure show a reduction of hygiene-relevant germs at least by a factor of 1000 after fermentation. The microorganisms studied include *Enterobacteriaceae* (*E. coli*, coliforms and *Salmonella*) as well as *enterococci* (e.g. *E. faecalis*). Investigations regarding the determination of relevant germs were carried out using plating method with different (non)selective nutrient media. The monitoring period for all important parameters such as substrate, metabolites, retention time and temperature of the biogas plant lasted for nearly one year.

AMP015

Virulence properties of panton-valentine leukocidin gene carrying foodborne *Staphylococcus aureus* strains

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Panton-Valentine Leukocidin gene carrying three methicillin-susceptible *Staphylococcus aureus* strains (M1, PY30C-b and YF1B-b) were isolated from different food samples in Turkey. These strains were characterized on the basis of MLST type, spa type, virulence factor gene contents, antibiotic susceptibilities against 21 antibiotics, biofilm formation, extracellular enzyme production, presence of protease and lipase genes. The genetic relatedness of the strains was determined by Pulsed Field Gel Electrophoresis analysis. All strains were found to be susceptible to all tested antibiotics and they were mecA and vanA negative. Three strains showed the same PFGE band pattern, ST152 clonal type and t355 spa type. Virulence genes; sea, seb, sec, sed, see, seg, seh, sei, sej, sek, sel, sem, sen, seo, sep, seq, seu, eta, etb, set1, geh and tst were not detected in the strains. All strains showed the positive results for alpha and beta-haemolysin genes, protease encoding genes (sspA, sspB and aur), lukED leukocidin genes. The strains were found to be non-biofilm formers and extracellular proteases were detected in skim milk and milk agar plates. Although the strains were geh gene negative, extracellular lipase production were observed in Tween80 containing medium but not in Tween20 containing medium. As a result of this study, in the extended spectrum, the characteristics and virulence properties of foodborne methicillin-susceptible *S. aureus* were described.

Reference:
Mert Sudagidan, Ali Aydin (2010). Virulence properties of methicillin-susceptible *Staphylococcus aureus* food isolates encoding Panton-Valentine Leukocidin gene. *International Journal of Food Microbiology* 138, 287-291.

AMP016

Multidrug and inducible clindamycin resistance in foodborne *Staphylococcus aureus* strains

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The presence of pathogenic and antibiotic resistant *Staphylococcus aureus* strains in food can cause important infectious risks for public health. In this study, 154 *S. aureus* strains isolated from 1070 food samples were examined on the basis of antibiotic susceptibility profiles against 21 antibiotics and inducible clindamycin resistance using D-test. Additionally, the presence of ermABC, mecA and vanA genes were investigated by PCR experiments. The antibiogram results indicated that 39 strains (25.3%) were resistant to three or more antibiotics defined as multidrug resistant (MDR). All strains were susceptible to vancomycin, oxacillin, cefoxitin and imipenem. Additionally, inducible clindamycin resistance was determined in 5 strains by D-test. In fact, only strain HE7A showed constitutive resistance to both erythromycin and clindamycin. PCR results demonstrated that none of the strains contained mecA and vanA genes, but all constitutive and inducible clindamycin resistant strains contained only ermC gene. Moreover, genetic-relatedness of MDR strains as well as inducible and constitutive clindamycin resistant *S. aureus* strains were determined using PFGE analysis. Some MDR strains from different sources showed 100% homology. PFGE analysis of the strains with inducible/constitutive resistance demonstrated two main clusters. By this study, the presence of MDR strains and inducible clindamycin resistance in foodborne *S. aureus* strains in Turkey were reported.

Reference:
Ali AYDIN, Karlo MURATOGLU, Mert SUDAGIDAN, Kamil BOSTAN, Burcu OKUKLU, Sebnem HARSA (2011). Prevalence and Antibiotic Resistance of Foodborne *Staphylococcus aureus* Isolates in Turkey. *Foodborne Pathogens and Disease* 8(1), 63-69.

AMP017

Nisin production in tofu by fermentation

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Tofu has a high nutritional content but on the other hand it can easily be spoiled. In Indonesia tofu production takes place mostly in small factories without an opportunity for pasteurization. Nisin production in tofu by fermentation with *Lactococcus lactis* ssp. *lactis* DSM 20729 may be a possibility to preserve tofu.
The fermentation of tofu cubes (2*2 cm) submerged in water was performed for two days. Two methods of detection of nisin in fermented tofu were tested and compared by using inhibition test (modified method of Pongtharangkul and Demirci 2004), and a liquid chromatography electrospray ionization tandem mass spectrometry method (LC-ESI-MS/MS), based on ISO/TS 27106:2009. The detection limit was significantly lower by using the inhibition test. The nisin content was 0.19 mg/kg in tofu and 0.06 mg/L in liquid substrate. In comparison to this the detection limit for nisin using LC-ESI-MS/MS was 0.34 mg/kg in tofu. Matrix calibration of the liquid substrate could not be carried out by LC-ESI-MS/MS, because the background noise of the matrix was too high.
Furthermore the production and distribution of nisin in tofu cubes was investigated. The nisin concentration on the surface of tofu was 2.64 mg/kg and so it was nine times higher compared to the interior of tofu. The optimum storage condition for fermented tofu was a combination of low temperature and low pH-value. An alternative to preserve tofu may be fermentation of tofu with *L. latis* ssp. *lactis*. Nisin concentration of complete tofu cubes could be successfully determined by using both methods. The inhibition test proved to be more sensitive for nisin detection in tofu.

Pongtharangkul T, Demirci A (2004) Evaluation of agar diffusion bioassay for nisin quantification. Appl Microbiol Biotechnol 65: 268-272
International Organization for Standardization: ISO/TS 27106 (2009) Cheese- determination of nisin A content by LC-MS and LC-MS/MS. Geneva, Switzerland

AMP018

Biogas from microalgae for sustainable electricity generation

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In this century biofuels will play an important role as fossil fuel alternatives. However, the long-term sustainability of large-scale biomass-based energy production chains is largely unknown.
Question: The current study evaluates the effect of nitrogen and phosphorous nutrient recovery on net energy ratio and land use.
Methods: Comparison of a prospective industrial scale microalgae production system with established maize agriculture. The functional unit was the delivery of 1.0 TWh of electrical energy using biomethane firing. Nutrient recovery was modeled by embedding anaerobic digestion as downstream processing step in the biomass production chains.
Results: The main finding was that maize-based biomethane electricity provision outperforms a prospective microalgae system in terms of net energy ratio, estimated at 4.9 and 3.2, respectively, when utilizing co-generated heat. In the absence of external fossil fuel input, the renewable maize- and microalgae-based systems would require 6.2·10^4 and 3.9·10^4 ha surface area, respectively.
Conclusions: Sustainability of microalgae-based biofuel production is not set by areal productivity or microalgal lipid content but rather by nutrient recovery, an important finding that requires prioritization in microalgae research.

AMP019

Pathatrix® Auto System: a Re-circulating Immuno-Magnetic Sepa-ration (RIMS) system to wet pool, concentrate and clean samples prior to food pathogen detection

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Pathatrix® Auto is a well established system which enhances the known non-disruptive technique of Immuno-Magnetic Separation (IMS). It selectively binds and concentrates target organism from many complex food matrices. The Pathatrix® Auto re-circulating system follows your normal enrichment procedure, and processes up to 60ml of sample. Magnetic beads with antibodies specific to your target organism capture cells from the entire sample, concentrating and cleaning your working sample prior to your chosen detection method.
This simple and automated technique takes just 15 minutes and boosts, by its cleaning nature, both the sensitivity and specificity of your detection method, reducing your false positives and PCR indeterminates. This technology is also unique as it enables wet pooling, a process where aliquots of up to 10 of your samples are pooled after enrichment, dramatically reducing the costs spent on the analysis. Time to results is reduced due to the concentration effect.
This paper highlights some key data, demonstrating the performance and robustness of the scientific approach behind the Pathatrix® Auto System.

AMP020

Genetic Engineering of *Escherichia coli* tolerant to biofuel

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A key challenge in the microbial production of biofuels (organic solvents) is to increase the tolerance of bacteria to the solvents. To find the target genes whose manipulation allow increasing tolerance to organic solvents (OSTs, organic solvent tolerances), we investigated candidate genes with knock-out mutants or with over-production-plasmids. The OSTs of *E. coli* are improved in mutants of two global regulators: MDR repressor *marR* and fatty acid metabolism regulator *fadR*. In fatty acid composition analysis, the OSTs mutants showed high ratio of saturated fatty acids to unsaturated fatty acids (SFA/UFA). The intracellular *n*-hexane accumulation levels of the OSTs mutants strain were lower than that of the wild-type. Furthermore, the additional introduction of a few genes with plasmids enabled the OSTs strains more improved OSTs.

AMP021

Accessing novel biosurfactant com-pounds from culti-vation based and environmental approaches

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Biosurfactant structures are of growing interest in detergent and pharmaceutical industry as they can be produced by microorganisms using renewable feedstock, are easily biodegradable and show interesting anti-microbial properties. A diverse range of microorganisms are known to produce these amphiphilic molecules but it is expected, that a huge variety combining the hydrophilic (mainly sugars, peptides or phosphor-bonds) and hydrophobic moieties (mainly fatty acids) are still undetected in nature.
Several screening methods have been optimized and developed to target different surfactant properties, such as surface activity, ionic properties, solubilization of hydrocarbons and emulsion capacities. These methods allow high throughput detection of strains producing yet unknown amphiphilic compounds from culture collections or libraries.
Interesting candidates have been found from environmental samples as exotic as Antarctic seal carcasses, peat-bog soils, deep-sea and sponge samples.
In order to detect producer strains missed by cultivation based methods, these screening assays are used to find surfactant producing activity directly from environmental samples, using metagenomic approaches.
Newly discovered clones, whether wildtype strains or heterologous expression clones producing novel surfactant compounds could lead to new non-pathogenic producer strains and further enable the production of surfactants with highly specific properties for industry.

ARV001

Characterization of the alternative heme synthase AhbNirJ1 from *Methano-sarcina barkeri*

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The cyclic tetrapyrrole heme serves as an essential cofactor in almost all living organisms. Whereas in eukaryotes and most bacteria heme is generated *via* the long known and well-characterized heme biosynthesis pathway, in some bacteria and all heme synthesizing archaea an alternative heme biosynthesis pathway is existent [1, 2]. Only recently, it was shown

that in sulfate-reducing bacteria such as *Desulfovibrio desulfuricans* the alternative route to heme proceeds *via* siroheme, 12,18-didecarboxysiroheme and iron-coproporphyrin III [3]. The enzymes catalyzing the respective transformations of the intermediates represent homologs to enzymes involved in heme d_1 biosynthesis and are also found in heme producing archaea such as the methanogen *Methanosarcina barkeri*.
Our studies are focused on the enzyme Ahb-NirJ1 from *M. barkeri* which functions as an alternative heme synthase by decarboxylating the two propionate side chains at positions C3 and C8 of iron-coproporphyrin III to the corresponding vinyl groups. Ahb-NirJ1 belongs to the Radical SAM enzyme family. All members of this family contain a characteristic CX_3CX_2C amino acid motif, which provides the cysteine ligands for a [4Fe-4S] cluster. Additionally, Ahb-NirJ1 also exhibits a second cysteine-rich motif at the C-terminus, which harbours another iron-sulfur cluster involved in catalysis or substrate binding.
Recombinant Ahb-NirJ1 from *M. barkeri* was produced in *E. coli* and purified in two chromatographic steps. The iron-sulfur clusters of the purified enzyme were reconstituted *in vitro* yielding an iron and sulfide content of 8.5 mol iron and 5.1 mol sulfide per mol Ahb-NirJ1. An *in vitro* enzyme activity assay was established. Using this assay we showed that Ahb-NirJ1 from *M. barkeri* catalyzes the decarboxylation of the two propionate side chains *via* a mono-vinyl intermediate.

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ARV002
Significance of archaeal nitrification in hypoxic waters of the Baltic Sea

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Marine oxygen deficient areas are sites of important microbially mediated transformations within the nitrogen cycle. In the Baltic Sea, hypoxic waters near the oxic-anoxic interface are considered a major nitrification zone in close proximity to subjacent sulfidic waters. Recent evidence indicates that *Archaea*, dominated by the abundant thaumarchaeotal subcluster GD2, and not *Bacteria* are the major ammonia oxidizers in this system. To date, little is known about nitrification activity in relation to the occurrence of GD2, its relevance for this process and the role of sulfide for nitrification, e.g. during local mixing or lateral intrusions of sulfidic water.
To approach these questions, we sampled pelagic redoxclines in the Baltic Sea, determined nitrification rates via ^{15}N incubations and the abundance of putative ammonia oxidizing thaumarchaeota via 16S rRNA based catalyzed reporter deposition fluorescence *in situ* hybridization (CARD-FISH). We spiked sample water with sulfide to determine its impact on nitrification activity and further elucidated the overall significance of archaea for nitrification by amendment with the archaeal inhibitor GC_7 to the ^{15}N incubations.
In the Gotland Deep in July 2011, the pattern of nitrification activity followed the distribution of thaumarchaeota and reached highest rates in the depth of maximal thaumarchaeotal abundance accounting for 122 nmol $N\ L^{-1}\ d^{-1}$ and 24% of total prokaryotic cell counts, respectively. Nitrification in water from this depth was still detectable after exposure to lower *in situ* concentrations of sulfide implicating a special adaptation to periodically occurring sulfide pulses. Supporting, we detected an elevated nitrification potential in water taken from a sulfidic depth. At Landsort Deep in June 2012, inhibition of archaea with GC_7 resulted in significantly reduced nitrification activity.
Our study indicates that ammonia oxidation in hypoxic waters of the Baltic Sea is mainly driven by thaumarchaeota. Their occurrence also in the anoxic, sulfidic water masses and the maintained nitrification potential point to special adaptations in this habitat with a potentially reduced sensitivity against hydrogen sulfide.

ARV003
Nitrosopumilus maritimus – analysis of its ultrastructure by electron microscopy

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Ammonia-oxidizing archaea (AOA) are widely distributed microorganisms in aquatic and terrestrial habitats. They catalyze the first and rate limiting step in nitrification (1). These chemolithoautotrophs belong to the phylum Thaumarchaeota (2), and they play a significant role in the global nitrogen cycle and contribute to primary production. As determined for *Nitrosopumilus maritimus* (3), their lipid compounds represent suitable biomarkers for determining $\delta^{13}C$ values of archaeal ammonia oxidizers without biosynthetic correction (4). Their genome has been analysed, revealing unique mechanisms for nitrification and autotrophy in this archaeal group of microorganisms (5). Ultrastructural data are basically not existing, or scarce (2). Because this is one of the smallest archaeal cells known today, with a diameter at around 200 nm and a length of about 500 to 600 nm, we started an approach for better understanding the ultrastructural organization of this archaeal cell by using a variety of up-to-date electron microscopical methods (6). This includes transmission electron microscopy of air-dried Platinum-shadowed cells for overall cell shape and detection of cell appendages, freeze-etching for analysis of cell envelope structures, and ultrathin sectioning, after cryo-preparation, for visualizing details of the interior of the cell and of the cell envelope.
The results will be presented and discussed in comparison to ultrastructural data from members of other archaeal taxa.

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7. We kindly acknowledge the support by a grant from the ERC project DARCLIFE to K-U Hinrichs

ARV004
S-layer proteins in pyrite-oxidizing Bacteria and Archaea - Structure, function and its limited application as taxonomic marker

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S-layer proteins can be found within Bacteria in some cyanobacteria as well as Archaea and in the latter case they often represent the only cell wall component. As a special feature, those surface proteins consisting of identical subunits arrange in a regular 2-D crystal with lattices that exhibit p1-, p2-, p3-, p4-, or p6-symmetry. [1]. Beside the symmetry, the lattice constants are often characteristic for an order or genus as it could be shown for the Sulfolobales and Thermoproteales [2]. In recent studies, the surface proteins of two *Acidithiobacillus* strains SP5/1 and HV2/2 (γ-proteobacteria) as well as *Metallosphaera sedula* TH2 and the *Sulfolobus metallicus* strains Kra23 and DK-I15 (Crenarchaea) were investigated. The main focus was on the structural characterization of the cells in general as well as the S-layer in detail via electron microscopy [3].

Furthermore, the involvement of the S-layer proteins in pyrite-oxidation by complexation of Fe(III) or surface attachment as well as their role as taxonomic marker was examined [4,5].

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ARV005

Intraspecies aggregation of *Sulfolobus* cells mediated by UV-induced pili

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Upon UV-stress, hyperthermophilic *Sulfolobus* species show highly induced expression of a gene cluster responsible for pili biogenesis. These UV-inducible pili (Ups-pili) are essential for cellular aggregation and subsequent DNA exchange between cells; abilities that increase the fitness of *Sulfolobus* cells. We therefore assume that transfer of DNA takes place in order to repair UV-induced DNA damages via homologous recombination [1, 2].
An interesting aspect about UV-induced cellular aggregation is its species specificity: all studied *Sulfolobus* species aggregate, but only with cells from the same species. To study the putative role of Ups-pili in this self-recognition we exchanged *S. acidocaldarius* pilin subunits with those from *S. tokodaii*. UV-induced cellular aggregation of these mutants was abolished. However, using fluorescence *in situ* hybridisation we could show that these strains are now in fact able to aggregate with *S. tokodaii* cells instead. By exchanging smaller parts of the subunits, we could moreover map a region in pilin subunit UpsA that seems to be primarily important for this specificity.
In addition, we studied the putative role of S-layer glycosylation in cellular recognition. To this end, high concentrations of sugars were added to *S. acidocaldarius* cells during aggregation assays. Addition of mannose resulted in reduced cellular aggregation. Related to this, we could show that deletion mutants of genes involved in glycosylation display altered aggregation behaviour. We therefore hypothesise that Ups-pili recognise glycosylated S-layer proteins on other cells, resulting in physical interactions between many cells. Small differences in pilin subunit UpsA and also composition of the glycan chains are thought to determine the species specificity in this process. The molecular mechanisms behind cellular recognition and interaction of *Sulfolobus* cells are currently being investigated.

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ARV006

A systematic study of the swimming behaviour of various species of the archaeal genus *Thermococcus*

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The archaeal genus *Thermococcus* contains a multitude of phylotypes, reported isolates, and many validly described species. The DSMZ collection momentarily has available 27 type species of these extremely thermophilic, obligate and facultative sulphur respiring *Archaea* from marine and terrestrial thermal environments. We started a systematic study of various species of this genus with the main focus on their swimming behaviour and with respect to some growth characteristics (growth in rich medium without sulphur; growth by sulphur respiration in minimal medium; growth at various temperatures) and their phylogenetic position via 16S rDNA sequencing. We are interested in the swimming behaviour because in many cases the original description of these species reports on the occurrence of cell appendages, which is taken as evidence for motility via archaeal flagella. A direct proof that these cell appendages indeed are functional flagella however is missing in nearly all cases. Only analyses at the actual growth temperature - and not at room temperature! - will allow to decide on the ability of these *Archaea* to actually swim. Motility analyses were done in rectangular glass capillaries using our so-called thermomicroscope, to reach observation temperatures of up to 95° C.
Data will be reported for some species using the DSMZ collection strains to show: (1) the temperature range and speed of swimming of *Thermococcus* species possessing flagella; (2) results for demonstrating thermotaxis; (3) that some species do not possess flagella; (4) that some of the strains analyzed are not phylogentically correctly placed; (5) the results of our physiological tests.

ARV007

Ultrastructural characterization of the hyperthermophilic Archaeon *Methanocaldococcus villosus*

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Question: The hyperthermophilic Archaeon *Methanocaldococcus villosus* was isolated from a shallow submarine hydrothermal system. Its up to 50 polar flagella were shown to be multifunctional organelles involved in motility, adhesion to surfaces, and formation of cell-cell contacts [1]. Using *M. villosus* as a suitable novel model organism, this study was set out to better understand not only the ultrastructure of flagella but the whole-cell architecture of Archaea with aid of various microscopic techniques.
Methods: Isolated flagella of *M. villosus* were biochemically characterized, and 3D-reconstructions from transmission electron micrographs were generated with the iterative helical real space reconstruction (IHRSR) algorithm [2]. To determine the cellular ultrastructure of *M. villosus*, cells were prepared for electron microscopy by high-pressure freezing/freeze substitution or conventional chemical fixation [3]. Specimens were investigated by transmission electron microscopy (TEM) and by focused ion beam scanning electron microscopy (FIB-SEM) with regard to substructures in the cell and anchoring of flagella in the membrane. Visualization of cells was done with Amira™.
Results: Electron microscopic analyses of the *M. villosus* cells revealed a densely packed cytoplasm containing distinct globules, 80-120 nm in diameter. Besides the well-preserved cell envelope, a submembraneous structure was identified, resembling bacterial chemoreceptor arrays. 3D-reconstructions of the cells showed that flagella were mostly anchored in proximity to these arrays. Exhibiting the filaments, a random mixture of at least two different symmetries was determined.
Conclusions: The different microscopic techniques used herein confirmed previous ultrastructural findings on flagella and cell envelope construction of the *Methanococcales* and gave new insights into the anchoring of flagella. Altogether, these findings enhance our general understanding of archaeal cell structures.

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ARV008

Growth by anaerobic sulfur dispro-portionation in acidianus ambivalens/ sulfurisphaera MC1 coculture

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The mechanisms of anaerobic disproportionation of elemental sulfur are not well known. The products are hydrogen sulfide and sulfate. In order to gain energy from this reaction low concentrations of free H_2S are required.
The thermoacidophilic and chemoautotrophic Archaeon *Acidianus ambivalens* grows either by sulfur oxidation with air or sulfur reduction with H_2 under anaerobic conditions. *Sulfurisphaera* MC1 is a heterotrophic strain. A coculture of *Acidianus ambivalens* and *Sulfurisphaera* MC1 incubated anaerobically with sulfur and CO_2 at 80 °C and pH 2,5 showed a constant growth with a doubling time of 120 h and a maximal cell density of 1 x 10^8 ml^{-1}.
Enzyme assays of total protein extracts showed sulfur oxygenase/reductase (SOR) activity (3U/mg oxygenase; 0,4 U/mg reductase) in cytoplasmatic and membrane fractions. In addition hydrogenase and sulfur reductase activities (0,2 U/mg) were found.
The results were supported by transcription analysis with RT real time PCR. The expression of the corresponding *sor*, *hyn* and *sre* genes was detected.
This coculture grows with enzymes of aerobic and anaerobic energy conservation involving the SOR, which was previously thought to be restricted to aerobic conditions. These results indicate that sulfur

disproportionation without oxygen is possible and that it might be a common mechanism of energy conservation in habits of volcanic origin, where anaerobic habits are common and sulfur is abundantly available.

ARP001

RNA-Seq analyses reveal tRNA processing events and the maturation of C/D box sRNAs and CRISPR RNAs

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The analysis of deep sequencing data allows for a genome-wide overview of the cellular small RNA pool, the "sRNome". Several archaeal organisms employ unusual RNA processing pathways during the maturation of transfer RNAs and C/D box sRNAs, key components of the translation machinery. We utilized RNA-Seq methodology in combination with biochemical studies to investigate some of these pathways in detail: (i) *trans*-splicing of tRNA halves in *Nanoarchaeum equitans* and (ii) C-to-U editing of tRNAs in *Methanopyrus kandleri*. The tRNA processing intermediates and the order of processing events could be identified [1]. In addition, these efforts yielded insights into CRISPR RNA and C/D box sRNA maturation. Circular C/D box sRNA molecules are abundant in archaeal cells and the production of anti-CRISPR RNAs was observed [2]. These studies exemplify the utilization of sequencing data for deducing small RNA maturation pathways.

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ARP002

Functional analysis of CRISPR-Cas systems in *Methanosarcina mazei* Gö1

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Methanosarcina mazei strain Gö1 belongs to the methylotrophic methanogens of the order *Methanosarcinales*, which have the most versatile substrate spectrum within the methanogenic archaea contributing significantly to the production of greenhouse gas [1,2]. The genome annotation published in 2002 [3] did not include the information on potential CRISPR loci in the archaeal model organism. We identified and analyzed the two main CRISPR loci in *M. mazei*. Both of them contain a conserved direct repeat of 37 nucleotides in length. The first CRISPR locus is flanked by a Cas type I-B system, whereas the second is flanked by a polycistronic operon encoding a RAMP module of CAS proteins (type III-B). Interestingly, based on sequence homology of already known Cas6 proteins, none of the loci obviously encode for the major endoribonuclease of crRNA maturation.
Here, we present the identification of two potential *M. mazei* Cas6 orthologs.
The biochemical characterization of the proteins will be presented and discussed.

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ARP003

Characterization of potential oligo-peptide encoding sRNAs in *Methano-sarcina mazei*

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Methanosarcina mazei is a methanogenic archaeon of high ecological importance due to its role in biogenic methane production. Recently we performed a deep sequencing analysis of the *M. mazei* Gö1 transcriptome in response to nitrogen availability (1), resulting in the identification 248 sRNA candidates in intergenic regions (IGRs). Detailed inspection of the sRNA sequences revealed a subset of 40 candidates which contain short ORFs potentially encoding small peptides (≤ 30 aa), which is supported by the fact that some of these are preceded by a consensus ribosome binding site. Interestingly, the majority of these ORFs is conserved in other *Methanosarcina* strains and, furthermore, also the flanking regions show conservation, indicating that some sRNAs might have a dual function as mRNA and regulatory sRNA.
To verify the transcription of previously selected sRNA candidates, we performed Northern-Blot analyses of *M. mazei* RNA. The obtained transcriptome data will be presented and discussed.

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ARP004

Characterization and genomic analysis of "*Methano-plasmatales*", a novel order of methanogens in termite guts and other habitats

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Recently, we showed that a deep-branching lineage of Euryarchaeota distantly related to the *Thermoplasmatales* represents a novel order of methanogens [1]. Member of the "*Methanoplasmatales*" occur in various environments, including marine habitats, soil, and also the intestinal tracts of termites and mammals. They were identified as methanogens by connecting the phylogeny of 16S rRNA genes to that of unknown *mcrA* gene sequences, a functional marker for methanogenesis, obtained from the same habitats and from methanogenic enrichment cultures from the hindguts of termites and millipedes. The highly enriched cultures of strains MpT1 and MpM2 obligately require both methanol and H_2 for methanogenesis. A draft genome of strain MpT1 contained all genes required for the reduction of methanol with H_2 as electron donor, whereas those involved in CO_2 reduction to the methyl level (hydrogenotrophic pathway) are apparently absent, explaining why strain MpT1 can neither reduce CO_2 to CH_4 nor disproportionate methanol to CO_2 and CH_4. The situation resembles that in *Methanomassilicoccus luminyensis*, the first isolate of this group [2], and the few other obligately H_2-requiring methylotrophic methanogens that have been isolated exclusively from intestinal tracts. Ultrastructural analysis indicate that strains MpT1 and MpM2 lack a cell wall and possess an unusual two-layered membrane system, which is present also in *M. luminyensis* and may be characteristic for members of the "*Methanoplasmatales*".

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ARP005

1.8 Å Crystal Structure of F_{420}-reducing [NiFe]-hydrogenase from *Methanother-mobacter marburgensis*

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The F_{420}-reducing [NiFe]-hydrogenase (Frh) is a cytoplasmic enzyme, which catalyzes the reversible reduction of coenzyme F_{420} with H_2 to $F_{420}H_2$. F_{420} is an important coenzyme in the central methanogenic pathway and functions as a hydride donor/acceptor. Here we present the X-ray structure of Frh, which is the first archaeal [NiFe]-hydrogenase structure, which belongs to class III [NiFe]-hydrogenases. In contrast to other [NiFe]-hydrogenases Frh forms a huge, cube-shaped complex with a molecular mass of 1215-kDa composed of twelve FrhABG heterotrimers. The tightly associated FrhABG protomers create only three small pores between cube interior and exterior. Each FrhABG protomer appears to perform catalysis independently such that the reason for the enormous size of Frh is still obscure. The fold of FrhA is highly related to that of the large subunit of known [NiFe]-hydrogenases but its smaller size significantly changes the routes for proton and molecular hydrogen to/from the [NiFe] centre. The [NiFe]-centre and the surrounding residues are highly conserved between Frh and the other known [NiFe]-hydrogenase family members. In the small subunit FrhG, the

proximal [4Fe4S]-cluster was bound to the protein with a unique coordination pattern with one aspartate and three cysteine residues. The C-terminal part of FrhG architecturally differs from the known [NiFe]-hydrogenases and rather corresponds to a ferredoxin fold containing two [4Fe4S]-clusters. Class III [NiFe]-hydrogenases posses an additional subunit termed FrhB. The 1.8 Å data clearly indicate the unusual conformation of FAD and the potential binding site of F_{420}. F_{420} oxidation activity of heterologously produced FrhB substantiated this hypothesis.

ARP006
Lrs14 transcriptional regulators influence biofilm formation and cell motility of Crenarchaea

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Archaea as others prokaryotes are found to thrive in a broad diversity of environments by forming biofilms. Despite it, the environmental cues and the molecular mechanisms driving archaeal biofilm development still remain to be characterized. The thermoaciphilic crenarchaeon *Sulfolobus* spp. have lately provided valuable information on the structural components that build up biofilms. Through comparative proteomic we recently revealed that transcriptional regulators belonging to the Lrs14-like proteins were one of the common biofilm-upregulated proteins within *Sulfolobus spp*. Here we hypothesize that this class of regulators might constitute a key regulatory factor during *Sulfolobus* biofilm development. Among the six *lrs14*-like genes encoded by *S. acidocaldarius*, the deletion of three of them revealed a remarkable altered biofilm phenotype. While Δ*saci1223*, Δ*saci1242* showed major dysfunctions to build biofilms, Δ*saci0446* exhibited a highly augmented EPS production, leading to a robust biofilm formation. Moreover, the expression of genes of the archaella and adhesive pili structures were found, respectively, down- and up-regulated in biofilm-cells of Δ*saci0446,* aditionally the mutant strain was shown to be non-motile. Through gel shift assays we further determined that saci0446 bound to the promoters controlling the expression of both cell surface structures. In addition, via genetic epistasis analysis using Δ*saci0446* as background strain we could identify one gene whose product is involved in the EPS biosynthetic pathway of *S. acidocaldarius*. These results imply the first step in understanding both the molecular mechanisms that underlie biofilm formation in Crenarchea and the functionality of the Lrs14-like proteins, an archaea-specific class of transcriptional regulators.

ARP007
Introduction of unusual amino acid modifications in the methyl-coenzyme M reductase of methanogenic archaea

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Methanogenic archaea conserve energy under anaerobic conditions by converting CO_2 and C_1-compounds into methane thereby forming an electrochemical gradient. The final metabolic step of methanogenesis is catalyzed by the methyl-coenzyme M reductase (MCR) which converts methyl-coenzyme M and coenzyme B to methane and the heterodisulfide CoM-S-S-CoB.
The MCR is a heterohexamer consisting of the three α, β and γ subunits ($\alpha_2\beta_2\gamma_2$) and two noncovalently bound F_{430} cofactor molecules (1,2). Crystal structure analysis revealed that the α-subunit (McrA) of *Methanothermobacter thermoautotrophicus* carries five co- or posttranslationally introduced amino acid modifications. Amongst these, 2-(*S*)-methylglutamine, 5-(*S*)-methylarginine and thioglycine have not been found in other organisms. Still, the methylation and thiolation pattern varies in McrA subunits of different methanogenic archaea species (3, 4). Moreover, biochemical studies showed that the unusual methylations result from a SAM-dependent mechanism (5).
Searching for archaea-specific open reading frames (ORFs) located in the proximity of the *mcr* operon (coding for the MCR subunits), two ORFs were identified downstream of the operon which encode proteins belonging to the Radical SAM enzyme family. These enzymes harbor a conserved cysteine motif CX_3CX_2C typical for [4Fe-4S] cluster-containing proteins. Based on their amino acid sequence the two identified Radical SAM enzymes can be grouped into two distinct classes. One, containing a further cysteine motif for a possible second iron-sulfur cluster, was found in strains harboring methylated arginine. The second class, characterized by an additional cobalamin-binding domain, was identified in archaea with methylated glutamine.
Representatives of each class were produced in *E. coli*, purified and further characterized. All in all, these proteins of unknown function might represent a new class of methyl-transferases providing new insights into the biochemistry of methanogenic archaea.

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ARP008
DNA as a nutrient storage molecule and the origin of polyploidy

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Polyploidy, the presence of multiple copies of the same chromosome in a cell, is common in prokaryotes, yet its evolutionary origin is unknown.
Halobacteriales are obligate hypersaline adapted Archaea and all investigated species were shown to be polyploidy (1). The model archaeon *Haloferax volcanii* has on average 30 chromosome copies in exponential phase, and was isolated from a hypersaline environment, where high salt concentrations preserve exogenous DNA (2). DNA is a readily available polymer containing the essential elements of life: carbon, nitrogen and phosphorus. Nitrogen and phosphorus are limiting nutrients in ecosystems and most dissolved phosphorus in the environment is often locked in DNA. Bacteria are capable of using DNA as a nutrient source (3), suggesting that it is an extracellular source of phosphate. These observations led to the hypothesis that an ability to uptake DNA from the environment first evolved to collect DNA as "food", and only later was recruited for DNA repair and as a mechanism for horizontal gene transfer.
Here we show that cells of *H. volcanii* grow using extracellular DNA as only source of phosphorus. Furthermore, we demonstrate by a real-time-PCR approach that during phosphate starvation, *H. volcanii* cells grow by cannibalizing extra chromosomes, which reduces chromosome copy number from 30 to 2. After reintroducing phosphate starved cells to medium with phosphate, the chromosome copy number increased up to 43 in the first 24 hours and reached a basal level of about 22 genome copies after six days. Therefore, polyploidy can provide a selective advantage under phosphorus-limited environmental conditions.
Altogether, these results indicate that *H. volcanii* utilizes extracellular and intracellular DNA as a phosphate storage polymer in parallel to store genetic information.

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ARP009
Cas6b, the crRNA processing endo-nuclease of CRISPR/Cas subtype I-B

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The CRISPR/Cas adaptive immune system found in many archaea and bacteria confers resistance against mobile genetic elements (e.g. viruses). The system is composed of an array ofclusteredregularlyinter-spacedshortpalindromicrepeats (CRISPR) andCRISPRassociated (Cas) genes. The hallmark of CRISPR systems are short CRISPR RNAs (crRNAs) that contain so-called spacer sequences that can be derived from a viral genome sequence. During future viral attacks the crRNAs are utilized to target DNA via base complementarity (1). A Cas protein family termed Cas6 processes the precursor crRNAs by cleaving repeat sequences. Different CRISPR/Cas subtypes employ different Cas6 variants. Common features of these endonucleases include the production of an 8 nucleotidé 5 -terminal repeat tag and the generation of 2′,3′-cyclic phosphates (2).
We characterized the related subtype I-B CRISPR/Cas systems of the archaeon *Methanococcus maripaludis* C5 and the bacterium *Clostridium thermocellum*. RNA-Seq analysis revealed *in vivo* processing and abundance

of crRNAs (3). We discovered a Cas6 homologue, termed Cas6b, and verified specificity for one of two repeat sequences found in *Clostridium thermocellum*. Mutagenesis and structure modeling of Cas6b identified two complementary histidine residues in its proposed catalytic site. Finally, comparative analyses of spacer-repeat-spacer processing by Cas6b revealed potential influences of spacer length and sequence on crRNA maturation and stability.

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ARP010

Insight into the function of the archaeal CRISPR/Cas I-A immune system

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The CRISPR (clusters of regularly interspaced short palindromic repeats) / Cas (CRISPR-associated proteins) system operates as an adaptive immune mechanism against a variety of mobile genetic elements (e.g. viruses or plasmids) in Bacteria and Archaea (1). The immunity is mediated by (i) the acquisition of foreign nucleic acids (protospacers), (ii) the processing of CRISPR transcripts into small RNAs (crRNAs) and (iii) the interference of future viral attacks via the targeting of protospacers by crRNAs and a multi-protein complex termed Cascade (Cas complex for antiviral defense). Due to the diversification of CRISPR systems, a classification into ten different subtypes was established (2).
Here, we present the analysis of CRISPR subtype I-A systems of the Crenarchaeote *Thermoproteus tenax*. RNA-Seq data analysis revealed the *in vivo* crRNA production and maturation for five of the seven identified clusters and an exceptionally high abundance of crRNAs in the cell. To facilitate a detailed analysis of the subsequent interference reaction, the respective *cas* genes of *T. tenax* were individually cloned, expressed and the reconstitution of Cascade with its native crRNA substrate was established (3). The archaeal Cascade complex is characterized by the presence of Csa5 and Cas8a in addition to the crRNA-binding domains Cas5 and Cas7 and a split of the helicase Cas3 into two discrete genes (Cas3, Cas3'). The protein-protein interactions of Cascade proteins were analysed by different chromatography techniques and their DNA and crRNA-binding activity were verified, substantiating the essential role of Cascade in the interference mechanism.

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ARP011

Pentose degradation pathways in the haloarchaeon *Haloferax volcanii*

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H. volcanii utilizes D-xylose and L-arabinose as a sole carbon and energy source. Both pentoses are shown to be degraded by an oxidative pathway to α-ketoglutarate (α-KG) as an intermediate. D-Xylose is oxidized by inducible xylose specific dehydrogenase (XDH) to D-xylonate followed by two dehydratase reactions (xylonate dehydratase, 2-keto-3-deoxyxylonate dehydratase) to yield α-KG semialdehyde that is oxidized to α-KG by α-KGSA dehydrogenase (Johnsen et al., 2009). The first step of L-arabinose degradation is catalysed by an inducible L-arabinose specific dehydrogenase (AraDH), which differs from XDH by kinetic properties and phylogenetic affiliation. AraDH belongs to a novel cluster of the extended short-chain dehydrogenase/reductase family (SDRe). Transcriptional analyses and deletion mutant experiments indicate that L-arabinoate conversion to α-KG involves the same set of enzymes as reported for D-xylonate degradation to α-KG. Further a bacterial like transcriptional regulator was identified, which is shown to activate genes of both D-xylose and L-arabinose degradation pathway.

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ARP012

Fructose degradation in the haloarchaeon Haloferax volcanii involves bacterialtype PEP-dependent PTS, 1-PFK and Class II FBA

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The halophilic archaeon Haloferax volcanii utilizes fructose as a sole carbon and energy source. Genes and enzymes involved in fructose uptake and degradation were identified by transcriptional analyses, deletion mutant experiments, and enzyme characterization. During growth on fructose, the gene cluster HVO_1495 to HVO_1499, encoding homologs of the five bacterial phosphotransferase system (PTS) components enzyme IIB (EIIB), enzyme I (EI), histidine protein (HPr), EIIA, and EIIC, was highly upregulated as a cotranscript. The in-frame deletion of HVO_1499, designated ptfC (ptf stands for phosphotransferase system for fructose) and encoding the putative fructose-specific membrane component EIIC, resulted in a loss of growth on fructose, which could be recovered by complementation in trans. Transcripts of HVO_1500 (pfkB) and HVO_1494 (fba), encoding putative fructose-1-phosphate kinase (1-PFK) and fructose-1,6-bisphosphate aldolase (FBA), respectively, as well as 1-PFK and FBA activities were specifically upregulated in fructose-grown cells. pfkB and fba knockout mutants did not grow on fructose, whereas growth on glucose was not inhibited, indicating the functional involvement of both enzymes in fructose catabolism. Recombinant 1-PFK and FBA obtained after homologous overexpression were characterized as having kinetic properties indicative of functional 1-PFK and a class II type FBA. From these data, we conclude that fructose uptake in H. volcanii involves a fructose-specific PTS generating fructose-1-phosphate, which is further converted via fructose-1,6-bisphosphate to triose phosphates by 1-PFK and FBA. This is the first report of the functional involvement of a bacterial-like PTS and of class II FBA in sugar metabolism of archaea.

ARP013

Effect of an overproduction of accessory Gvp proteins on gas vesicle formation in *Haloferax volcanii*

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Gas vesicles are proteinaceous, gas-filled structures, increasing the buoyancy of cells. In *Halobacterium salinarum* PHH1, their formation is driven by the p-vac region consisting of fourteen genes arranged in two clusters, *gvpACNO* and *gvpDEFGHIJKLM*[1]. The *gvpFGHIJKLM* transcript leads to essential accessory proteins, present in minor amounts in the exponential growth phase. It has been suggested that they play a role in early steps of gas vesicle formation[2]. Except for GvpK, the accessory proteins GvpF through GvpM are present in small amounts in gas vesicle preparations[3]. It is not clear whether these proteins are components of the gas vesicle structure or whether they are required during the process of formation. In this study we analysed the effect of an overproduction of the accessory Gvp proteins on gas vesicle formation. An overexpression of *gvpG*, *gvpH* or *gvpM* in addition to p-vac inhibited gas vesicle formation in *Haloferax volcanii* transformants, but large amounts of all other acessory proteins had no effect. GvpM has a tendency to aggregate, as shown by Western analysis and *in vivo* using a fluorescent GvpM-GFP fusion. In search for proteins neutralizing the inhibitory effect of GvpM, we analysed transformants overexpressing another Gvp protein in addition to GvpM. GvpH, GvpJ or GvpL were able to suppress the inhibitory effect of GvpM on gas vesicle formation, but GvpG was unable to do so. Western analyses of HM and JM transformants with antisera directed against GvpH or GvpJ showed larger protein bands in addition to the GvpH or GvpJ monomers suggesting an interaction of GvpH-GvpM and GvpJ-GvpM. A reason for the inhibition of gas vesicle formation could be that these proteins act like a sink and prevent the formation of Gvp aggregates required to form the gas vesicle structure. A balanced amount of these proteins appears to be important to form gas vesicles.

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ARP014

Insights into molecular differentiation of a novel Antarctic haloarchaeal biofilm

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Although biofilm formation is the predominant lifestyle of microorganisms in nature it is only poorly understood in archaea. A screening and characterization of several haloarchaea strains and isolates demonstrated that the ability for adhesion and biofilm formation is a widely spread property. Compared to the other haloarchaea tested the novel Antarctic isolate DL24 showed the strongest adhesion in a fluorescence based adhesion assay. The cells are able to adhere on glass and plastic surfaces forming biofilms of densely packed multi-cell layers with tower-like macrocolonies up to 50 μm in height. Since the underlying mechanisms are unknown we used a proteomic approach for an initial investigation of molecular factors involved in biofilm formation of isolate DL24.
Static liquid cultures of isolate DL24 were cultivated for 28 days in petri dishes before planktonic cells and cells of biofilms were harvested separately. Comparative protein analysis using SDS-PAGE yielded differential protein patterns for planktonic and adherent cells. Subsequent MS-analyses identified 801 different proteins in biofilm cells and 678 proteins in planktonic cells. In comparison 573 proteins were present in both protein sets while 228 proteins were associated with biofilm cells and 105 proteins were solely detected in planktonic cells. The specific proteins were categorized according to the cluster of orthologous groups of proteins (COG) and provided first insights into the biological processes contributing to biofilm formation of isolate DL24. Evidence was found for an adjustment of energy metabolism from aerobic to anaerobic energy conversion. A number of transcriptional regulators as well as components of signal transduction systems involved in information processing were detected. Furthermore several biofilm specific proteins related to the cell envelope, cell surface structures and biofilm matrix were identified.
Our results demonstrate that biofilm lifestyle goes along with fundamental molecular rearrangements on the protein level affecting diverse biological processes in haloarchaea.

ARP015

Development of β-lactamase as a tool for studying *cis*- and *trans*-acting gene regulation in *Methanosarcina acetivorans*

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The use of reporter gene fusions to assess cellular processes such as protein targeting and regulation of transcription or translation has been established for decades in bacterial and eukaryal genetics. Fluorescent proteins or enzymes resulting in chromogenic substrate turnover, like β-galactosidase, have been particularly useful for microscopic and screening purposes. However, such methodology is not available for strictly anaerobic organisms due to the requirement of molecular oxygen for chromophore formation or color development. We have developed β-lactamase from *Escherichia coli* (encoded by *bla*) in conjunction with the chromogenic substrate nitrocefin into a reporter system usable under anaerobic conditions for the methanogenic archaeon *Methanosarcina acetivorans*. By replacing the *E. coli* signal peptide for excretion with that of a putative flagelin from *M. acetivorans* we could demonstrate β-lactamase activity in supernatants of *M. acetivorans* cultures. Fusing this hybrid gene to the promoters of the catabolic methanol ($mtaCB1_p$) or methylamine ($mtmCB_p$) methyltransferase 1 genes, respectively, resulted in growth substrate-dependent expression of *bla*, which could be visualized anaerobically on agar plates. Furthermore, a series of fusions comprised of the constitutive *mcrB* promoter ($mcrB_p$) and variants of the synthetic tetracycline-responsive riboswitch (Tet-RS) was created to characterize its influence on translation initiation in *M. acetivorans*. One Tet-RS variant resulted in a more than 10-fold tetracycline-dependent regulation of *bla* translation, which is well in the range of regulation by naturally occurring riboswitches. Thus, the $mcrB_p$-Tet-RS-*bla* fusion represents the first solely *cis*-active, i.e. factor-independent system for controlled gene expression in Archaea.

ARP016

Translational regulators in *Haloferax volcanii*

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The translational regulation of gene expression is a general feature in bacteria, eukarya as well as in archaea. In the halophilic archaeon *H. volcanii* a growth phase-dependent translational regulation of 6 % of all genes, could be revealed [1].
Many transcripts were translated with under-average efficiency in the exponential or stationary phase, indicating that their translation might be induced in response to a changing stimulus. Therefore, the translatoms for various stress conditions were investigated.
It was found that under low salt conditions (0.7 M NaCl) about 5.8 % and under high salt conditions (4 M NaCl) only 0.4 % of the analysed genes show translational regulation. Deprivation of phosphate or nitrogen revealed that 2.4% and 6.6 % of the analysed genes are regulated on translational level. Among these genes several genes for cell division and ribosomal genes as well as a translation initiation factor were found to be up regulated. The regulation of these selected genes could be additionally verified by Northern blot analyses.
Recent data revealed that the 5´- and 3´-UTRs are necessary to transfer translational control from native transcripts to a reporter transcript [2]. Therefore, the UTRs of some of the genes observed to be regulated are used in a reporter gene system with dihydrofolate reductase (DHFR) to analyse the translational regulation to prove the concept of translational control independently.
Additionally the *H. volcanii* genome was bioinformatically screened for putative RNA-binding proteins. To gain insight into their function the respective genes for selected proteins were deleted and a conditional overexpression system in *H. volcanii* for those genes was generated. Analyses of the isolated proteins, e.g. the search of binding partners, are currently under way.
To sum up, all the different approaches finally confirm the strong influence of regulation on translational level in *H. volcanii*.

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ARP017

Haloferax volcanii, a first prokaryotic species that does not use the Shine Dalgarno mechanism for translation initiation at all

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It was long assumed that translation initiation in prokaryotes generally occurs via the so called Shine Dalgarno (SD) mechanism, which includes base-pairing between a SD motif about 5 nt upstream of the start codon and the 3'-end of the 16S rRNA to position the ribosome on the mRNA. Recently it became clear that translation initiation in prokaryotes is more heterogeneous and also leaderless transcripts and transcripts with 5'-UTRs without SD motifs can be efficiently translated. A bioinformatic survey of 162 available prokaryotic genomes showed that the fraction of genes preceded by a SD sequence ranges from about 15% to more than 90%, dependent on the phylogenetic group. In archaea, in 16 of 21 species less than 50% of all genes are preceded by a SD motif [1].
An *in silico* analysis of the genome of *Haloferax volcanii* revealed that only very few genes are preceded by a SD motif. Therefore, we decided to analyze whether SD motifs are involved in translation initiation in haloarchaea *in vivo*. The 5'-UTR of the *sod* (superoxide dismutase) gene, which contained a very extended SD motif, was fused to a reporter gene, and a consecutive series of mutants was constructed that matched the SD consensus motif from zero to eight positions. The translational efficiencies for these mutants were determined by quantitative Western blot and Northern blot analyses under various conditions. All mutants exhibited almost the same translation efficiency, irrespective of growth rate and salt concentration. Complete replacement of the SD motif by several other unrelated sequences also did not abolish translation initiation, but, in contrast, even enhanced the translation efficiency for one mutant. To sum up, our results prove that translation initiation in *H. volcanii* is totally independent from SD/aSD complementarity.

Therefore *Haloferax volcanii* is the first prokaryotic species that does not make use of the SD mechanism at all.

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ARP018

Biosignatures of methanogenic archaea from Siberian permafrost investigated by confocal Raman micro-spectroscopy for astrobiological research

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Methanogenic archaea from Siberian permafrost have proven to be interesting candidates for potential past or present life on the Martian subsurface. Several novel strains were recently isolated from the Lena Delta (Russia), which show an anaerobic chemolithotrophic metabolism generating methane gas. They are also remarkably resistant against desiccation, osmotic stress, low temperatures and starvation [1]. Previous studies show that these methanogens are able to survive simulated thermo-physical Martian conditions [2] as well as high doses of UV-C and ionizing radiation, making them exciting candidates for astrobiological research. As part of the "Biology and Mars Experiment" (BIOMEX) project [3], this study aims to gain a deeper insight into the biosignatures of methanogenic archaea by means of confocal Raman microspectroscopy (CRM), a new and powerful approach for describing such signatures. Its high sensitivity allows the in situ detection of unique biomolecules within living systems, making it more informative than other approaches such as mass spectrometry [4,5]. Furthermore, the ExoMars Mission will include a Raman spectroscope among its analytical instruments and this work will therefore contribute to a Raman biosignature database that will help interpret future data from Mars. In this first-ever Raman study on methanogenic archaea, we have investigated the biosignatures of the strain Methanosarcina soligelidi SMA21 over its growth. Results show spectral changes, evidencing changes in the chemical composition of the cell over time, as well as the synthesis of biogenic products. In addition, these changes in the cell composition correlate with morphological modifications.

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ARP019

Molecular characterization of a novel lytic virus infecting the methanogenic archaeon *Methanobacterium formicicum*

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Due to the limited availability of fossil fuels and the detrimental effect on climate of their use, sources for renewable energy are becoming increasingly important. One such source is biogas, produced via anaerobic syntrophic degradation of biomass and consisting mainly of methane, which is exclusively formed by methanogenic archaea in a process called methanogenesis. Even though there has been progress in understanding the biogas process on a molecular scale, methane digesters in most parts are still considered as black boxes. Furthermore, the role of phages as one key factor affecting microbial population dynamics has been demonstrated for some habitats but the relevance of phages for the structure and dynamics of archaeal communities in methane digesters is not understood at all. In a first effort to gather insight into the role of phages in the biogas process, a mesophilic hydrogenotrophic methanogen was isolated from a biogas plant in Germany and found to be a *Methanobacterium formicicum* strain. *M. formicicum* is abundant in methane digesters and thus presumably relevant for the biogas process. From the same biogas plant a novel lytic archaeophage infecting *M. formicicum*, designated Drs3, was also isolated. Transmission electron microscopy and preliminary analysis of the viral dsDNA suggests that Drs3 belongs to the *Siphoviridae* family.

ARP020

Insights into the metabolism of thermophilic methane oxidizing archaea

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The anaerobic oxidation of methane (AOM) controls the emission of the greenhouse gas methane from the ocean floor. AOM is performed by microbial consortia of anaerobic methanotrophic archaea (ANME) associated with partner bacteria. Until recently, AOM has solely described in cold habitats (methane seeps and sulfate-methane transition zones), and only the ANME-2 type has been enriched *in vitro*. In hot vent sediments from the Guaymas Basin we discovered AOM at elevated temperatures. The early enrichments hosted a diverse ANME-1 dominated microbial community. In long-term cultivation with methane as sole energy source we produced sediment-free AOM enrichments, which we characterized by molecular techniques and physiological experiments.
Enrichments obtained in 37°C incubations are characterized by dense microbial aggregates of ANME-1 and partner bacteria of the Seep-2 cluster. At 60°C a different ANME-1 subgroup forms aggregates with HotSeep-1, a novel, deeply-branching strain of Deltaproteobacteria. Thee Guaymas enrichments are to our knowledge the first known cultivable ANME-1 types. Moreover they grow faster than low-temperature adapted ANME-2.
The ANME in the studied AOM enrichments are obligate methane oxidizers - a methane production on methanogenic substrates such as hydrogen, methanol and methylamines has been excluded. Yet only sulfate has been identified as electron acceptor for thermophilic AOM. In ^{13}C-labeling experiments, analyzed by single cell and bulk lipids we showed that those ANME assimilate only inorganic carbon. Consequently, ANME-1 should be described as obligate methane-oxidizing chemoorganoauto-trophic archaea. We successfully isolated HotSeep-1, the bacterial partner of ANME-1. Current physiological tests and genomic analysis of HotSeep-1 will enhance our understanding of the functioning of AOM.

ARP021

The uncultivated SM1 Euryarchaeon- uncovering its genetic potential

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Although detectable in most environmental settings, Archaea are usually minor components of a microbial community and dominated by a large and diverse bacterial population. The SM1 Euryarchaeon, however, predominates a sulfide-containing aquifer by forming subsurface biofilms that contain a very minor, sulfate-reducing bacterial fraction (5-10%)[1,2]. These unique biofilms are delivered in high biomass to the spring outflow. As soon as the biofilms reach the surface and are therefore in contact with oxygen, the SM1 Euryarchaeon seems to selectively seek interaction with sulfide-oxidizing bacteria to form the so-called "string-of-pearls communities". In these communities, the bacterial partner and the SM1 Euryarchaeon are present in almost equal abundance, pointing at a "real" partnership and possibly at a symbiotic/syntrophic relation and allowing a growth and persistence even in surface waters.
However, possible metabolic functions of the SM1 Euryarchaeon remain speculative, but may be responsible for the environmental success of this organism. In order to understand the genetic potential of this unusual euryarchaeon, we performed metagenomics on biofilm samples using a combined 454 pyrotag and Illumina paired-end sequencing approach that generated approximately 300 Gb of data. Using hybrid assembly approaches we tried to reconstruct the genome of the SM1 Euryarchaeon and used state-of-the-art genome annotation software tools to predict metabolic capabilities of this highly interesting, uncultivated archaeon.

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ARP022

The uncultivated SM1 Euryarchaeon- uncovering its ultrastructure

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Although detectable in most environmental settings, Archaea are usually minor components of a microbial community and dominated by a large and diverse bacterial population. The SM1 Euryarchaeon, however, predominates a sulfide-containing aquifer by forming subsurface biofilms that contain a very minor, sulfate-reducing bacterial fraction (5-10%)[1]. These unique biofilms are delivered in high biomass to the spring outflow.
On its surface, the SM1 Euryarchaeon carries highly unusual cell surface appendages, the hami [2]. One hamus is composed of three basic filaments that are twisted around each other and form barbwire-like prickles at periodic distances. At the distal end, the hamus carries a nano-sized grappling hook, representing a perfect tool for the attachment to biotic and abiotic surfaces.
The hami seem also to be responsible for the formation and maintenance of the archaeal biofilm, mediating strong cell-cell contacts between the archaea. On this poster, we will present novel insights into the ultrastructure of the SM1 Euryarchaeon, its surface appendages and the SM1 biofilm. For the analysis, we used different techniques, such as transmission and scanning electron microscopy which revealed fascinating structural details of this unusual archaeon and its biofilm from nano- to micron-level.

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ARP023

Archaea in terrestrial freshwater springs

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Terrestrial freshwater springs are essential water resources for diverse ecosystems. Approximately 10% of all terrestrial freshwater springs are sulfidic aquifers that are the nutritional basis for a high biomass of microorganisms involved in sulfur cycling. Recently, the so-called SM1 Euryarchaeon was found to predominate the microbiome of different sulfidic freshwater sites near Regensburg, Germany[1]. This finding represents an interesting basis for studying novel Archaea in natural settings and gives evidence for an important role of Archaea in freshwater systems. In the current study we present investigations on the microbiomes of sulfidic and non-sulfidic terrestrial freshwater springs with main emphasis on Archaea. Different parameters like oxygen, sulfide, temperature and pH were measured on site when collecting several liters of the freshwater of each spring. After filtration, DNA was extracted from the filters and screened for archaeal and bacterial signatures. Quantification of 16S rRNA gene signatures allowed the conclusion that terrestrial aquifers harbor an indigenous archaeal community. Further on, 16S ribosomal databases have been revisited and a specific freshwater group within the Euryarchaota that included the SM1 Euryarchaeon was identified. In sum, Archaea seem to play a major role in the microbiome of freshwater springs, although their role and capacities remain to be explored.

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ARP024

Biofilm formation by haloarchaea

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A fluorescence based live-cell adhesion assay was used to examine biofilm formation by twenty different haloarchaea, including species of *Halobacterium*, *Haloferax* and *Halorubrum*, as well as novel natural isolates from an Antarctic salt lake. Thirteen of the twenty tested strains significantly adhered (P-value < 0.05) to a plastic surface. Examination of adherent cell layers on glass surfaces by differential interference contrast, fluorescence and confocal microscopy showed two types of biofilm structures. Carpet-like, multi-layered biofilms containing micro- and macrocolonies (up to 50 μm in height) were formed by strains of *Halobacterium salinarum* and the Antarctic isolate t-ADL strain DL24. The second type of biofilm, characterised by large aggregates of cells adhering to surfaces, was formed by *Hfx. volcanii* DSM 3757^T and *Hrr. lacusprofundi* DL28. Staining of the biofilms formed by the strongly adhesive haloarchaeal strains revealed the presence of extracellular polymers, such as eDNA and glycoconjugates, substances previously shown to stabilize bacterial biofilms. For *Hbt. salinarum* DSM 3754^T and *Hfx. volcanii* DSM 3757^T, cells adhered within one day of culture and remained viable for at least 2 months in mature biofilms. Adherent cells of *Hbt. salinarum* DSM 3754^T showed several types of cellular appendages that could be involved in the initial attachment. Our results show that biofilm formation occurs in a surprisingly wide variety of haloarchaeal species.

ARP025

Investigation of homologous heme-based sensor proteins from *Methanosarcina acetivorans*

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The predicted sensor kinases MA0863 and MA4561 from *M. acetivorans* share high sequence homology. Both proteins consist of two alternating PAS and GAF domains fused to a C-terminal kinase domain. MA0863 contains one additional PAS domain at the N-terminus. MA4561 was formerly predicted to be a multidomain phytochrome-like photosensory kinase that binds open-chain tetrapyrroles. While we were able to show that recombinantly produced and purified MA4561 protein does not bind any known phytochrome chromophores, UV-vis spectroscopy revealed the presence of a heme tetrapyrrole cofactor. In contrast to many other known cytoplasmic heme-containing proteins, the heme was found to be bound covalently via one vinyl side chain to cysteine 656 in the second GAF domain. Interestingly, the redox state of the heme cofactor has a direct influence on the kinase activity. First UV-vis spectroscopic data revealed that recombinant MA0863 also binds heme in its second GAF-domain. Despite its high sequence homology, MA0863 is different from MA4561 as it owns an in-frame amber-codon (TAG) in its second PAS domain. This codon encodes for pyrrolysine in *M. acetivorans*, whereas it is used as a stop-codon in most other organisms. When MA0863 is expressed in *E. coli*, a terminated protein-variant is produced due to the stop-codon. Therefore we aim to expand the genetic code of *E.coli* by integration of genes for the biosynthesis and incorporation of pyrrolysine to produce a read-through full-length protein. As MA0863 and MA4561 are most likely sensor kinases of two-component systems, we furthermore intend to elucidate the signal transduction pathway that possibly involves the transcriptional activation of genes encoding methyltransferase/corrinoid fusion proteins involved in methylsulfide metabolism. The function of the heme cofactors in both sensor proteins and the pyrrolysine in MA0863 is discussed.

ARP026

Rotor subunits with and without signal sequences in archaea: on the evolution of the insertion of ATP synthases into the membrane

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Archaea have an ATP synthase that is distinct from the ATP synthase found in bacteria, chloroplasts and mitochondria and the organelles of eukaryotes (1). It is composed of two motors connected by a central stalk and two peripheral stalks that are mounted to a collar perpendicular to the membrane (2). The membrane-embedded rotor consists of a ring of subunits, the *c* subunits. We have purified the *c* subunits from members of the *Euryarchaeota*, *Crenarchaeota* and *Nanoarchaeota* by chloroform/methanol extraction. MALDI-TOF-MS analysis revealed that the protein of *Ignicoccus hospitalis*, *Thermoproteus neutrophilus* and *Sulfolobus*

acidocaldarius is smaller than suggested from their DNA sequence. Molecular studies proved that the primary transcript was as deduced from the DNA data, but N-terminal sequencing of the encoded protein revealed that the N-terminus was apparently absent in the mature protein. These data show the presence of a signal sequence that was also confirmed by bioinformatic approaches. In contrast *c* subunits from *Methanosarcina acetivorans*, *Pyrococcus furiosus, Methanocaldococcus jannaschii* and *Nanoarchaeum equitans* do not have a signal sequence. A model on the insertion of the ATP synthase into the membrane in the distinct groups of archaea and its evolutionary implication is presented.

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ARP027

Biofilm formation of the thermo-acidophilic archaeon *Sulfolobus acidocaldarius* in different cultivation systems

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Biofilms are microbial aggregates which usually accumulate at solid-liquid interfaces. The cells are embedded in a self-produced matrix of extracellular polymeric substances (EPS). In contrast to bacteria, knowledge of archaeal biofilms is still limited. Due to their adaptation to extreme environments, archaeal biofilms may be relevant for applications in biotechnology. The aim of this project is to find out if biofilms of *S. acidocaldarius* DSM639 may be used for biotechnological applications, e.g. for biocatalysis under harsh conditions in flow-through biofilm systems. *S. acidocaldarius* is a thermoacidophilic archaeon with optimal growth at 76 °C and pH 3.
In first experiments, *S. acidocaldarius* was grown in polystyrene 96-well microtiter plates under static conditions to characterize biofilm formation under different growing conditions and to evaluate optimal biofilm production conditions for subsequent experiments. The quantification of planktonic cells and biofilm was done by measurement of optical density of planktonic cells, crystal violet staining of biofilm and DAPI cell staining of planktonic cells and biofilm. Furthermore, pH change was monitored and biofilm morphology was analyzed by microscopic methods. With these methods significant biofilm formation was detected when the cells were grown with Brock medium, NZ-amine and dextrin under aerobic conditions at pH 3 and 76 °C for three days.
For biotechnological applications a flow-through system with constant media flow will be established. As suitable models for studies on biofilm formation three different flow-through systems were chosen, including a tubing reactor system, a flow-cell as well as a stainless steel biofilm reactor with glass slides as substratum. The biofilms are visualized by microscopic techniques and different dyes to characterize biofilm formation and composition of the EPS. These experimental setups will be used to analyze the stress response on *S. acidocaldarius* biofilms induced by toxic solutes, e.g. butanol and hexane.

BRV001

A signal transduction system tying cell shape with cell divisvion in *E. coli*

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The Rcs phosphorelay system is a complex 6-member signal transduction system that is conserved in all enteric bacteria. Upon perturbing the cell envelope the Rcs system launches a multi-faceted response that targets central processes, including cell division, motility, capsule synthesis, virulence and biofilm formation. Despite extensive work, our understanding of the signal(s) sensed, the way information gets transduced to downstream Rcs components and the role of the system in coordinating cellular responses remains incomplete. Based on our recent chemical genomics screen in *E. coli*,we have developed and pursued further hypotheses on different aspects of Rcs signaling.
First, we have established that a number of chemical and genetic perturbations that strongly induce the Rcs system lead also to changes in cell shape, i.e. cells becoming rounder. RcsF, the furthest upstream component of the system is required for sensing these changes. RcsF, an outer membrane lipoprotein, is partially surface exposed in vegetatively growing cells, but when cells start changing shape, RcsF relocates to face the periplasm and triggers the Rcs response. We have shown that changes in cell shape, RcsF "flipping" and Rcs activation happen concomitantly. As RcsB, the transcriptional regulator and output of the Rcs system is known to regulate expression of the cell division regulator FtsZ, our findings indicate a novel path of coordinating cell shape and cell division. Furthermore, we have established that RcsF transduces the signal to downstream Rcs components by relieving inhibition of the phosphorelay by the essential inner membrane protein IgaA. We have preliminary evidence that IgaA plays a role in sensing and/or regulating peptidoglycan remodeling. Thus, the complexity of the Rcs system seems to enable the system to integrate multiple signals and thereby tie together central envelope processes.

BRV002

The antibiotic tropodithietic acid can replace acylated homo-serine lactone as global gene regulator in *Phaeobacter* sp. DSM 17395

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The ability to sense information through cell-cell signaling depending on cell densities within the population and to respond by a coordinated gene expression is defined as bacterial quorum sensing. This is often regulated by acylated homoserine lactones (AHLs). The production of AHLs is widely distributed within the *Roseobacter* clade, one of the most common groups of marine bacteria, which can make up to 25 % of the total bacterial community (Gram *et al*., 2002; Wagner-Döbler and Biebl, 2006). In *Phaeobacter* sp. DSM 17395, our roseobacter model organism, it was previously shown that expression of the genes coding for production of the antibiotic tropodithietic acid (TDA) is strongly reduced in AHL synthase and AHL regulator mutants. Exogenous TDA, however, can reconstitute the wild-type expression level of the *tda* genes in the AHL synthase deficient mutant (Berger *et al*., 2011).
In this study we used dual-labeled microarrays to compare complete transcriptomes of the wild type of strain DSM 17395 with that of AHL deficient mutants to investigate which genes are AHL regulated. Furthermore, we analysed the transcriptome of the AHL synthase deficient mutant grown with exogenous TDA to investigate whether TDA regulates not only its own gene expression but has also influence on other genes. The microarray data showed that 20 % of the genome, coding, e. g. for motility or antibiotic biosynthesis, is AHL regulated. Interestingly TDA can reconstitute the wild-type genome expression in the AHL synthase deficient mutant. To our knowledge we show here for the first time that an antibiotic is regulating gene expression like an AHL on a global genome level and is therefore acting as a putative signaling molecule in quorum sensing.

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BRV003

A GFP promoter fusion library for the study of *Salmonella* biofilm formation and the mode of action of biofilm inhibitors

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To study the gene expression and regulation in *Salmonella* biofilms, we constructed a library of ~100 different GFP-promoter fusions of important *S.* Typhimurium biofilm genes, based on literature and in-house data. These include genes involved in biofilm regulation, matrix production, quorum sensing, metabolic genes and genes involved in motility and c-di-GMP synthesis and degradation. This library is currently being used to study the mode of action of biofilm inhibitors, to localize gene expression in biofilms, to study strain differences and to identify anti-biofilm targets.
For the mode of action studies, the effect of *Salmonella* biofilm inhibitors on the GFP expression is measured in time. As such we can quickly identify the effect of the compounds on specific biofilm-related processes. Results with one of our biofilm inhibitors indicate that this compound inhibits the expression of *csgD*, encoding the master regulator of *Salmonella* biofilms, and its downstream genes *adrA* and *csgB*. Furthermore we were able to

pinpoint a number of upstream regulators responsible for the *csgD* downregulation.
Biofilms are known to be heterogeneous. Therefore we used the gene reporter library to study spatial differences in expression within rdar colonies by fluorescence imaging and FACS analysis. Clear location dependent differences in gene expression were found amongst others for *csgD*, *fimA* and *flhD*.
Since *in situ* biofilms can exist of more than one microbial species, it is essential for the future identification of new inhibitors to identify anti-biofilm targets which are of importance both in monospecies and multispecies biofilms. Therefore we compared by FACS analysis the expression of the promoter fusions in monospecies *Salmonella* biofilms and multispecies biofilms containing *Salmonella*. Some genes showed a high and similar expression in monospecies and multispecies biofilms (without being expressed in free-living cultures) and therefore form good targets for new anti-biofilm strategies.
Remarkable differences in the biofim formation capacity of S. Typhimurium strains exist. We used the reporter fusion library to compare the gene expression in biofilms of strains ATCC14028 and SL1344. We could explain the lower biofilm formation capacity of SL1344 by a lower expression of *mlrA* (as previously described by Garcia, *et al*., 2004) and further attribute this to a lower expression of rpoS.

BRV004

Cyanobacteria's specific features: non-standard circadian clocks and plenty of antisense RNAs

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Cyanobacteria possess a circadian clock system that consists of mainly three proteins: KaiA, KaiB and KaiC control daily cycles of gene expression and chromosome compaction. Our previous analyses revealed that complex formation between Kai proteins and, therefore, their stoichiometry is essential in maintaining robust circadian oscillations (Brettschneider et al., 2010). Thus, it is puzzling that the chromosome of many cyanobacteria contains multiple *kai*-gene copies including the cyanobacterium *Synechocystis* sp. PCC 6803, a model organism for photosynthesis and industrial applications.
For a biochemical characterisation, we performed phosphorylation assays of the three *Synechocystis* KaiC proteins revealing that KaiC1 phosphorylation depends on KaiA, whereas KaiC2 and KaiC3 autophosphorylate independently of KaiA. This was confirmed by *in vivo* interaction studies, which demonstrate that only KaiC1 interacts with KaiA suggesting that the *kaiABC* gene cluster could regulate time-keeping of cellular processes in *Synechocystis* whereas the other Kai proteins may have different functions.
Interestingly, our global transcriptomic analyses of light-dark synchronised *Synechocystis* cultures indicate a rather light-driven than a circadian regulated pattern in global gene expression. We detected several small RNAs encoded at the *kai* gene loci but antisense to *kai* genes which might be involved or even interfere with circadian regulation. Besides several other studies, we have already shown how small RNAs can influence the temporal regulation of gene expression (Dühring et al, 2006; Legewie et al. 2008, Schmiedel at al., 2012). Thus, regulation by antisense RNA might be a fundamental mechanism for the temporal coordination of gene expression in cyanobacteria.

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BRV005

Targeted decay of small RNA GlmZ by RNase E adaptor protein YhbJ and inhibition by sRNA mimicry

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In *Escherichia coli*, feedback control of glucosamine-6-phosphate (GlcN6P) synthase GlmS is mediated by sRNAs GlmY and GlmZ that act in a hierarchical manner to attune the expression of *glmS* to the concentration of its enzymatic product GlcN6P. Exclusively sRNA GlmZ can base-pair with an inhibitory structure within the *glmS* 5'UTR that masks the ribosome binding site. Base-pairing leads to activation of the *glmS* transcript and subsequently to synthesis of GlmS which catalyses formation of GlcN6P, an essential precursor for cell wall biosynthesis [1,2,3,4]. However, GlmZ is subject to processing by RNase E which removes the base-pairing nucleotides and initiates degradation of GlmZ. Intriguingly, sRNA GlmZ *per se* is not a substrate for RNase E and processing of GlmZ *in vivo* and *in vitro* requires protein YhbJ. The novel sRNA binding protein YhbJ binds GlmZ and acts as an RNase adaptor protein by promoting RNase E dependent processing of GlmZ. The second sRNA GlmY acts indirectly to activate *glmS* expression, i.e. by sequestration of adapter protein YhbJ and concomitant stabilization of GlmZ. This regulation is crucial under GlcN6P limitation and these conditions induce accumulation of GlmY by a post-transcriptional mechanism [5]. Thus, we show that the molecular mechanism for regulation of *glmS* expression within the GlmYZ cascade depends on an RNase adapter protein that recruits GlmZ to its processing machinery. Further, we propose an anti-adapter function for sRNA GlmY that is exerted upon GlcN6P starvation and leads to activation of *glmS* by sequestration of adapter protein YhbJ from GlmZ [6]. Thus, YhbJ might be the first of many dedicated proteins programming a general ribonuclease for processing of a specific target (s)RNA.

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BRV006

A small RNA represses expression of the TlpB chemotaxis receptor by targeting a homopolymeric G-repeat

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The intense study of Helicobacter pylori, one of the most prevalent human pathogens, has contributed much to the understanding of bacterial virulence mechanism as well as its genomic diversity. In contrast, only a few transcriptional regulators have been described in the small Helicobacter genome and so far almost nothing is known about the role of post-transcriptional gene regulation in this pathogenic Epsilonproteo-bacterium. However, based on a differential RNA-seq approach we have recently discovered ~60 small RNA (sRNA) candidates in H. pylori strain 26695, including potential regulators of cis- and trans-encoded target mRNAs [1]. The functional characterization of abundant sRNAs and antisense RNAs will now provide new insights into riboregulation in H. pylori.

Here we focus on the very abundant and highly conserved sRNA HPnc5490, which is induced under acid stress and accumulates in late exponential growth phase. Bioinformatics-based predictions indicated that HPnc5490 could directly bind to a G-repeat far upstream in the 5' UTR of tlpB mRNA, which encodes for one of the four chemotaxis receptors of H. pylori. TlpB is assumed to play a role in pH-sensing, pH taxis, quorum-sensing as well as in the inflammatory response upon infection in mice [2, 3]. Analyses of transcriptome and proteome changes upon deletion of HPnc5490 revealed down-regulation of tlpB on the mRNA as well as protein level and complementation of the HPnc5490 deletion mutant restored tlpB repression. Furthermore, complementation with several mutant sRNAs as well as in-vitro gel-shift assays confirmed a direct interaction between the C/U-rich terminator loop of HPnc5490 and the G-repeat within tlpB mRNA. Interestingly, the G-repeat varies in lengths among diverse Helicobacter strains and represents one of the phase-variable homopolymeric repeats in H. pylori. Moreover, in-vitro structure probing and toeprinting experiments indicated that down-regulation of tlpB via HPnc5490 is rather based on structural rearrangements, transcript destabilization or transcription attenuation than on inhibition of translation initiation. Overall, our results confirm HPnc5490 as a first example of a trans-encoded sRNA regulator in H. pylori and reveal HPnc5490 as the first example of a sRNA which targets a homopolymeric repeat.

BRV007

Novel response on central metabolism perturbations in *Corynebacterium glutamicum*: Instantaneous stop of phosphortransferase system-mediated sugar uptake

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The Gram-positive bacterium *Corynebacterium glutamicum* co-metabolizes most carbon sources such as glucose and sucrose. Uptake and phosphorylation of these two sugars are mediated by the phosphotransferase system (PTS). The PTS of *C. glutamicum* possesses specific EII-permeases for each glucose and sucrose, encoded by *ptsG* and *ptsS*, respectively. Deletion of *pgi,* encoding the enzyme phosphoglucoisomerase, blocks the first step of glycolysis and directs the glucose-derived carbon flux towards the pentose phosphate pathway. *C. glutamicum* Δ*pgi* grows poorly with glucose as the glucose uptake is drastically reduced in order to avoid excessive accumulation of glucose 6-phosphate (G6P). Even though G6P is also formed in the sucrose metabolism, *C. glutamicum* Δ*pgi* grows well with sucrose as a sole carbon source. Surprisingly, addition of glucose to sucrose-cultivated *C. glutamicum* Δ*pgi* cells immediately arrested their growth. In detail, presence of glucose caused a strong inhibition of sucrose uptake and a drastic reduction of the *ptsS*-mRNA amounts in *C. glutamicum* Δ*pgi*.
Here we investigated the mechanisms underlying the response of sucrose-cultivated *C. glutamicum* Δ*pgi* upon glucose addition. The transcriptional regulator SugR acts as a repressor of the PTS encoding genes in *C. glutamiucm*. Indeed deletion of *sugR* in *C. glutamicum* Δ*pgi* abolished the negative effect of glucose on *ptsS*-expression, but was not sufficient to recover sucrose utilization and uptake in the presence of glucose. Kinetic analyses of the sucrose uptake inhibition by glucose in *C. glutamicum* Δ*pgi* revealed that inhibition took place within the first 15 s after glucose addition and could not be prevented by inhibitors of both transcription and translation. As expected, deletion of *ptsG* in *C. glutamicum* Δ*pgi* abolished all negative effects of glucose on sucrose uptake. While addition of the non-PTS substrate maltose to *C. glutamicum* Δ*pgi* inhibited both growth on and uptake of sucrose, these negative effects of maltose addition were not observed for the EII_{Glc}-deficient *C. glutamicum* Δ*pgi* Δ*ptsG*. The results presented here show that the *ptsG* encoded EII_{Glc} is part of a novel mechanism for instantaneous inhibition of EII_{Suc}-mediated sucrose uptake upon central metabolism perturbations in *C. glutamicum*.

BRV008

High-resolution comparative transcriptome analysis of multiple *Campylobacter jejuni* strains

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Campylobacter jejuni is the leading cause of bacterial gastroenteritis in humans to date. Comparison of multiple strains revealed a high genetic and phenotypic diversity among strains. However, only little is known about differences in transcriptome organization and gene expression as well as small RNA (sRNA) repertoires of *Campylobacter*. Here we present the first comparative primary transcriptome analysis of four *C. jejuni* isolates, including a genome-wide, automated prediction of transcriptional start-sites (TSS). This automated procedure will facilitate transcriptome analyses for a wider range of organisms and will also allow for a comparative TSS annotation among different growth or stress conditions. Our comparative dRNA-seq approach showed that most TSS are conserved in the four *Campylobacter* strains, but revealed also single-nucleotide-polymorphism (SNP)-dependent promoter usage and strain-specific sRNA expression patterns. Furthermore, *Campylobacter* possesses a minimal CRISPR-system, which seems to be active in only some strains. Besides genome-wide TSS maps of four *C. jejuni* strains, our study provides new insights into transcriptome diversity and riboregulation not only in *Campylobacter* species but also in related pathogens.

BRP001

A novel mechanism of osmoregulation: The GgpS protein in *Synechocystis* sp. PCC 6803 is regulated by salt dependent binding of nucleic acids

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The cyanobacterium *Synechocystis* sp. PCC6803 is moderately tolerant against salt stress. The main response to salt stress is the synthesis of the compatible solute glycosylglycerol (GG), the key enzyme in its biosynthesis pathway being the glycosylglycerolphosphate synthase (GgpS). GgpS activity is downregulated under low salt conditions, however the mechanism of regulation was unknown. We found that the inhibitory principle is binding to nucleic acids, including both DNA and RNA in a noncompetitive manner. We furthermore showed that binding of GgpS occurs by electrostatic interaction between positively charged amino acid residues of the protein with the negatively charged backbone of the nucleic acid. The binding affinity was found to be very high under low salt conditions whereas it was abolished upon salt addition.
We investigated homologous GgpS proteins from different cyanobacteria and found that the GgpS from *Synechococcus* sp. PCC 7002 also binds to nucleic acids. In contrast, the GgpS from *Synechococcus* sp. WH8102 showed no interaction. Interestingly the positive amino acid residues which we postulated to be involved in nucleic acid binding in *Synechocystis* based on experiments using covalent modification are missing in *Synechococcus* sp. WH8102. For further investigation of the DNA binding sites in the *Synechocystis* GgpS we replaced several positive amino acids which are putatively involved in the nucleic acid interaction. These variants were analyzed by electromobility shift assays for their interaction with nucleic acids. We found four variants with a reduced binding ability to nucleic acids. The functional properties of these variants in catalysis (GG synthesis) and regulation (salt dependent activation) will be discussed.

BRP002

Dynamic interaction between a sensor kinase and its periplasmic accessory protein mediates signal recognition

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Two-component signal transduction systems (TCS) are the main mechanisms by which bacteria sense and respond to environmental stimuli [1]. TCS typically consist of a sensor kinase (SK) and a response regulator (RR). The SK autophosphorylates upon detecting an inducing cue and transfers the phosphoryl group to its cognate RR which now promotes changes in cellular physiology or behavior [1]. To keep the TCS in balance, the RR gets dephosphorylated intrinsic or due to the phosphatase activity of the SK [1]. However, the mechanistic details about the precise signal integration and transfer remain still unknown [2].
The Cpx-envelope stress system is a well established TCS composed of the membrane-bound SK CpxA, the cytosolic RR CpxR and in addition of the accessory protein CpxP [3]. Factors that cause cell envelope stress as e.g. pH stress, salt stress and misfolded proteins induce the Cpx-TCS [3]. The accessory protein CpxP inhibits autophosphorylation of CpxA and supports the degradation of misfolded pilus subunits [3]. Previous functional and structural studies suggest that CpxP might be involved in sensing misfolded pilus subunits, pH, and salt [4]. By employing membrane-SPINE [5] and bacterial two-hybrid system, we were now able to demonstrate the direct physical interaction between CpxP and CpxA *in vivo* in *Escherichia coli*. Furthermore, our data show that salt and misfolded pilus subunits induce the release of CpxP from CpxA. Release of CpxP from CpxA is assumed to result in the autophosphorylation of CpxA [3]. Thus, our data assign CpxP as the sensor for theses specific Cpx-inducing stimuli. In sum, our combined results lead to a deeper insight into the signal recognition in TCS in general.

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BRP003

Regulation of anaerobic hydrocarbon-degradation in *Aromatoleum aromaticum* EbN1

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Aromatic hydrocarbons like benzene, toluene, ethylbenzene and xylenes (BTEX) are used in large amounts in industrial processes and therefore lead to widespread environmental pollution. Because of their high solubility in water, these compounds easily reach groundwater or deep layers of soil, where they encounter anoxic conditions. The genome-sequenced β-proteobacterium *Aromatoleum aromaticum* is capable to degrade the hydrocarbons toluene and ethylbenzene as well as different phenolic compounds anaerobically [1,2]. The genes coding for the enzymes of anaerobic toluene metabolism are induced co-ordinately in the presence of toluene, whereas those coding for the enzymes of anaerobic ethylbenzene metabolism are induced sequentially in the presence of ethylbenzene and the intermediate acetophenone, respectively. Three operons coding for two-component regulatory systems were identified in the genome sequence of *A. aromaticum* as possible candidates for affecting the induction of all toluene-catabolic genes (*tdiSR*), the induction of ethylbenzene-catabolic genes by ethylbenzene (*ediSR*) and the induction of acetophenone-catabolic genes by acetophenone (*adiRS*). Our main aim is to clarify the mechanisms involved in discrimination of these very similar aromatic substrates.
The two-component regulatory systems AdiRS, EdiSR und TdiSR are composed of sensory histidine kinases and response regulators with considerable similarity to each other. All components are predicted to be soluble cytoplasmic proteins. In our studies we show that the *adiRS* operon is indeed involved in the acetophenone-dependent induction of gene expression. The function of these gene products was investigated by genetic and biochemical studies. Moreover, the predicted acetophenone-sensing histidine kinase (AdiS) and the corresponding response regulator (AdiR) were overproduced in *E. coli* and purified. First results on their biochemical properties will be shown.

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BRP004

Understanding structural interactions within two component systems

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Bacteria have to deal with a large variety of environments and are urged to adapt most quickly to changing living conditions. The largest groups of regulatory signal transduction systems enabling this adaptation are two component systems (TCS). TCSs are typically composed of a transmembrane sensor Histidine Kinase (HK) and a cytoplasmic Response Regulator (RR) which facilitates the genetic response. Significant structural sequence identities have been found among TCSs. Nevertheless, TCSs comprise high specificity between the respective HK and RR pairs.
Structural insights are fundamental for understanding protein-protein interactions. Hence, a structural homology model of the cytosolic part of the HK CpxA and its RR CpxR was developed, based on the HK-RR co-crystal structure solved by the group of Marina et al (1). Putative interaction sites were derived from this model and investigated by MSPINE (2). The identified interaction sites are currently under further *in vivo* analysis by BACTH, especially by creating mutants with substitutions at the positions of the respective interacting amino acids. All in all, our investigations contribute to a better understanding of the signal transduction mechanisms within TCSs.

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BRP005

The FMN-riboswitch controlled gene *lmo1945* (*ribU*) from *Listeria monocytogenes* encodes a functional riboflavin/ roseo-flavin transporter

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The riboflavin auxotrophic bacterial pathogen *Listeria monocytogens* is unable to synthesize riboflavin (vitamin B_2) *de novo*. In the genome sequence of *L. monocytogens* a single FMN riboswitch was identified directly upstream of the gene *lmo1945*. The putative gene product of *lmo1945* is similar to the energy dependent riboflavin transporter RibU from *Bacillus subtilis*. *L. monocytogens* depends on riboflavin uptake and thus RibU is essential for growth and a potential target for novel antibiotics. Riboflavin dependent gene expression has been shown by Mansjö and Johansson, however, the function of *lmo1945* has not been studied [1].
The recombinant expression of *lmo1945* in a riboflavin auxotrophic *ribB*-(riboflavin synthase EC 2.5.1.9) - strain of *B. subtilis* with deleted *ribU* restored the ability of this strain to grow on LB-agar plates without riboflavin supplementation. In a second experiment *lmo1945* was expressed in *B. subtilis* with intact riboflavin synthesis genes but deleted *ribU*. These cells showed a higher sensitivity to roseoflavin, compared to the control containing the plasmid not carrying *lmo1945*. These results show that RibU from *L. monocytognes* is not only transporting riboflavin into the cells but also the antibiotic analog roseoflavin.

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BRP006

The distribution of microbial products of Xanthomonas and Clostridia on phopshorylated hosts

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The distribution of a particular microbial product is directly related to the phosphorylation potential of the host substrate.This relationship can be determined both by microscopy methods and by optical sensor methods. Electron microscopy and confocal light microscopy show a direct connection between the nature and extent of phosphorylation processes within the host substrate and the structure and cellular efficiency of the microbial product. This in itself affects the extent of distribution of the microbial product on the host substrate and the distribution affects the ability of the product to be manipulated to other intracellular and intercellular purposes.The products of both Xanthomonas and Clostridia can be manipulated from this starting point.Therefore to develop a statistical method of determining exactly what the distribution is of the microbial product on the phosphorylated host by RNA analysis methods can tell us would be very advantageous for the investigator wishing to proceed from the microbial product to a derivative would be a useful and important step.

BRP007

Three Fur orthologs in context of zinc transport regulation in *Cupriavidus metallidurans*

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The β-proteobacterium *Cupriavidus metallidurans* is a model organism to study bacterial resistance to transition metals. Survival of this bacterium in environments with high concentrations of metals ions such as Zn^{2+}, Cd^{2+}, Co^{2+} and Ni^{2+} is made possible by an arsenal of metal efflux systems. Zn^{2+} is a structure element in zinc finger motifs and acts as cofactor for some essential catalytic enzymes like alcohol dehydrogenase or carboxypeptidase. Therefore, zinc uptake into the cells is also necessary, and this function is performed by a battery of redundant und rather unspecific secondary import systems, namely ZupT, CorA1-3, and PitA.
To maintain zinc homeostasis by a balance of uptake and efflux reactions, zinc-dependent regulation is required. Regulators of the *fur*-family act as transcriptional repressors that bind metal ions. Most prominent is the Fur repressor for iron uptake, but other proteins might interact with zinc (Zur), manganese (Mur) or nickel ions (Nur). *C. metallidurans* contains three members of the Fur protein family, designated FurA, FurB and FurC, all encoded by the two chromosomes of this bacterium. FurA and B are more closely related to *E. coli* Fur and FurC more to Zur. FurA seems to be the

major iron uptake regulator in *C. metallidurans*. In this context *in silico* analysis, physiological characterization of Δ*fur* strains were performed to analyze the function of the three Fur proteins in *Cupriavidus metallidurans*.

BRP008

RNase E and RNase J regulate quorum sensing in *Sinorhizobium meliloti*

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We compared by microarrays the wild type *Sinorhizobium meliloti* Rm2011 to isogenic mutants with mini Tn5 insertions in the genes for RNase J (*rnj*) and RNase E (*rne*), respectively [1,2], and found overlapping and specific effects. qRT-PCR analyses confirmed up-regulation of *ndvA* important for glucan export and down-regulation of the flagella genes *flaA* and *flgB,* and revealed growth-phase dependent differences for the chemotaxis-related genes *cheR* and *mcpW* in both mutants. Since the last four genes are under the control of quorum sensing, the production of acyl-homoserine lactones (AHLs) was measured [3]. The two RNase mutants produced higher AHL amounts, although neither the amount of mRNA of the AHL synthase gene *sinI* nor its translation was increased. However, the stability of a SinI-eGFP fusion protein was higher in the mutants, suggesting that increased SinI stability may account for the increased AHL production. Interestingly, essentially no AHLs were detected in cultures of the complemented RNase E mutant, which shows up-regulation of *rne* at mRNA level. Consistently, induced overexpression of RNase E in the wild type led to lower expression of the SinI-eGFP fusion protein. The 5'-UTR of *sinI* was sufficient for the observed, specific effect, strongly suggesting that RNase E controls the expression of *sinI* in *S. meliloti*.

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BRP009

Characterization of iron-binding sites in the redox sensor protein HbpS from streptomycetes

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HbpS (heme-binding protein of *Streptomyces*) is an extracellular octamer-forming protein, which is secreted *via* the twin-arginin translocation pathway. It acts as an accessory protein of the two-component system SenS-SenR from the cellulose degrader *Streptomyces reticuli*. HbpS senses redox stress signals in form of toxic concentrations of iron ions or other redox active compounds. At these conditions it induces autophosphorylation of the membrane-embedded sensor kinase SenS, which in turn phosphorylates the response regulator SenR. This activates the transcription of genes involved in anti-oxidative stress response (i.e. *cpeB* encoding for the mycelia-associated catalase-peroxidase CpeB). Under non-stressing conditions HbpS inhibits SenS autophosphorylation, leading to down-regulation of the HbpS-SenS-SenR signalling pathway [1,2,3]. By sequence analysis iron-binding motifs (D/E-X-X-E) have been identified in HbpS; these are also well conserved among many uncharacterized HbpS-like proteins of different Gram-positive as well as Gram-negative bacteria [3]. D/E-X-X-E motifs are found in several iron-binding proteins that are involved in sensing, transport and storage of iron ions [4]. Analysis of the high resolution crystal structure of HbpS revealed that the mentioned motifs are located either within the protein core or on the protein surface. In order to get more insights into their functional role, a set of mutant proteins were generated and analyzed *in vivo* and *in vitro*. As the HbpS-SenS-SenR system has been postulated as a model, the obtained results are important not only for streptomycetes but also for other bacteria with ecological, biotechnological and medical relevance.

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BRP010

Redox signalling mediated by the extracellular heme-binding protein HbpS from *Streptomyces reticuli*

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HbpS is an extracellular protein that specifically interacts with the membrane-embedded sensor kinase SenS and modulates its autophosphorylation. While under non-stressed conditions HbpS inhibits SenS autophosphorylation, under oxidative-stressing conditions activates it. SenS in turn phosphorylates the response regulator SenR that activates the transcription of genes (*i.e., cpeB* encoding a catalase-peroxidase) involved in the anti-oxidative stress response in the bacterium *Streptomyces reticuli* [1]. The high resolution crystal structure of HbpS revealed an octomeric assembly which is required for interaction with SenS, and hence for its activation or inhibition, respectively [2]. Using mutagenesis, FRET, CD spectroscopy, fluorescence spectroscopy and site-directed spin labelling combined with pulse electron paramagnetic resonance (SDSL EPR) spectroscopy, we demonstrated that iron-mediated oxidative stress induces conformational changes within HbpS [1,3]. These events are responsible for the up-regulation of the HbpS-SenS-SenR signalling cascade. Furthermore, we have shown that the catalase-peroxidase CpeB protects HbpS *in vivo* from H_2O_2-mediated oxidative attack. Moreover, CpeB provides *Streptomyces reticuli* with a non-stressed environment, in which freshly synthesised HbpS proteins down-regulate the HbpS-SenS-SenR cascade. As homologues to the HbpS-SenS-SenR system are widespread in different bacteria with ecological, biotecnological and medical relevance, it has been proposed as a bacterial model of a redox-signalling pathway.

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BRP011

A sRNA in *Rhodobacter sphaeroides* which is processed upon $^1O_2$ exposure

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Exposure to oxygen and light generates photooxidative stress by the bacteriochlorophyll *a* mediated formation of singlet oxygen ($^1O_2$) in the facultatively photosynthetic bacterium *Rhodobacter sphaeroides* (1). By the use of differential RNA-sequencing five regulatory small RNAs (sRNAs) have been identified, which featured an altered expression or processing in response to $^1O_2$. Most likely these sRNAs influence gene expression at the post-transcriptional level for the benefit of the stress response. The most abundant of the identified sRNAs is RSs0682, which is processed upon $^1O_2$ exposure. The putative RNA processing site has been determined by 5'RACE. Interestingly, the 3'-segment of the full-length sRNA displays the processed sRNA and is highly conserved between different *Rhodobacterales* species (2). Moreover the RSs0682 processing was not observed in an *hfq* deletion strain of *Rhodobacter sphaeroides* and the interaction of the full-length sRNA as well as the 3'-segment with 3xFLAG-Hfq was proven by co-IP experiments. This indicates an important role of the RNA chaperone (3). One major interest is the identification of potential targets of RSs0682. To investigate the impact of the sRNA an RSs0682 overexpression strain will be used for Microarray analysis as well as proteome analysis. Deletion of RSs0682 was not possible, which might be due to an essential function of this particular sRNA. In addition, the search for putative targets will be completed by a genome wide screening for potential interactions of RSs0682 with mRNAs by the use of IntaRNA. Finally we plan to identify the RNase that catalyses the RSs0682 cleavage. Using knock-out strains it was already shown in vivo that the processing is RNaseIII- and RNaseJ-independent.

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BRP012
Inhibition of quorum sensing in Gram-Negative bacteria by staphylococcal compounds

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Bacteria use signal molecules to regulate population density in a process of bacterial communication called quorum sensing. This process plays critical roles in regulating various physiological activities, including production of antibiotics, secretion of virulence factors, pigments as well as formation of biofilms. It is found that various bacteria are able to secrete compounds for interfere quorum sensing signals in other bacteria. In our previous study on coinfection of *Staphylococcus* and *Pseudomonas aeruginosa*, we observed that *P. aeruginosa* could repress the growth of pathogenic staphylococcal *species* but not of nonpathogenic staphylococcal *species* by respiratory inhibitors. Meanwhile, to our surprise, some strains of the nonpathogenic staphylococcal *species* exhibit unknown compounds to interrupt the function of quorum sensing-controlled factors in gram-negative bacteria. Supernatants of several staphylococcal *species* were collected and incubated with *P. aeruginosa* and *Serratia marcescens* to test the activity of pigment inhibition. To purify the compounds, stationary-phase cell free supernatant from a strain belonging to *Staphylococcus sp.* was dialyzed with 3-kDa molecular weight cut-off membrane, extracted with methanol-acetone mixtures, adsorbed on XAD-16 resin and fractionize by elution buffer and further separated with HPLC. Effects of compounds on quorum sensing co-ordinate factors expression in Gram-negative bacteria were tested with *P. aeruginosa, S. marcescens* and *Vibrio harveyi* and determined by HPLC and Tecan reader. Physical analysis by molecular weight cut-off membrane demonstrated that the molecular weights of compounds are below 3 kDa. Moreover, compounds resist alkaline and acid pH, high temperature and proteinase K treatment, which might exclude compound as peptides. Purified compounds show activity of inhibition of the red prodigiosin pigment in *S. marcescens*, bioluminescence in *V. harveyi* and the blue-green pyocyanin and biofilm formation in *P. aeruginosa*. This study finds new compounds secreted by *Staphylococcus* that inhibit quorum- sensing-regulated behaviors in gram-negative bacteria species. The compounds have the potential to be further developed as new therapy against the pathogenicity of *S. marcescens* and *P. aeruginosa*.

BRP013
Dissection of the molecular requirements for Hfq binding and RNase E processing of *trans*-encoded small RNAs in *Escherichia coli*

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Trans-encoded small RNAs constitute a major class of post transcriptional regulators in *Enterobacteriaceae*. In Gram negative bacteria such as *Escherichia coli*, most small RNAs execute their downstream function via RNA chaperone Hfq (1). Two sRNA molecules, GlmY and GlmZ act as key regulators of a pathway involved in cell wall biosynthesis mediating glucosamine-6-phosphate (GlcN6P) homeostasis. When GlcN6P levels are low in the cell, GlmZ base-pairs with the *glmS* mRNA (GlcN6P synthase) in an Hfq dependent manner and activates translation by unmasking the Shine Delgarno sequence (2, 3, 4). GlmY, on the other hand, is an Hfq independent sRNA. Upon an increase in GlcN6P level, GlmZ is cleaved by RNase E with the help of an adaptor protein YhbJ, abolishing its base-pairing ability, thereby leading to down regulation of the entire cascade. This effect is counteracted by GlmY, via sequestration of YhbJ (5). The two sRNA molecules are highly homologous and share many structural features. The molecular components required for Hfq binding by small RNAs are not completely understood. In this study, hybrids of the two small RNAs were created to determine the discriminating factors leading to preferential binding of only one of the sRNAs to Hfq. In addition, the same hybrids were tested in RNase E assays to verify which regions within the secondary structure could be responsible for selective processing by RNase E.

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BRP014
PvpRA - a molecular switch controlling virulence and persistence in *Pseudo-monas aeruginosa*

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The opportunistic pathogen *Pseudomonas aeruginosa* is responsible for chronic infections in the lungs of patients suffering from cystic fibrosis. During long-term adaptation to the lung environment *P. aeruginosa* switches from acute infection into a state of chronic, persistent infection. A key factor controlling this lifestyle transition is the ubiquitous bacterial second messenger cyclic-dimeric-GMP (c-di-GMP). Generally, high intracellular levels of c-di-GMP favor persistence and suppress acute virulence phenotypes. However, little is known about the molecular mechanisms underlying this c-di-GMP-based switch from virulence to persistence.
We have identified the *pvpRA* (***P****seudomonas* **v**irulence/**p**ersistence) operon, which encodes key regulators that control the switch from acute infection towards persistence in *P. aeruginosa*. *pvpR* encodes a HTH transcriptional regulator of the XRE-family, and activates its own expression as well as expression of PvpA, a small, soluble protein with no predicted homology. Although the operon organization and gene annotation of *pvpRA* is reminiscent of toxin/anti-toxin systems, unbalanced expression of PvpA is not toxic for the cells, but rather induces a SCV (**s**mall **c**olony **v**ariant) morphotype, a hyper-adherent, auto-aggregative morph associated with immune system resistance and persistence of infection. PvpA acts by increasing c-di-GMP levels via a pathway that specifically involves one of the major *P. aeruginosa* diguanylate cyclases, WspR. The *pvp* operon is under the control of the Gac/RsmA pathway and is therefore expressed at high cell densities during stationary phase. These findings suggest that WspR is indirectly influenced by the global regulatory Gac/RsmA cascade. Furthermore, western blot data show that PvpA also negatively influences the expression of T3SS components. Our data suggest that PvpRA is an important part of the network controlling the transition from acute to chronic infection states in *P. aeruginosa*.

BRP015
The sRNA repeat RSs0680a-d modulates C1-metabolism in *Rhodobacter sphae-roides* under specific stress conditions

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When dealing with environmental changes bacteria employ a network of regulatory factors that are based mainly on proteins but also on regulatory RNAs. A special type of such a regulatory response is the post-transcriptional gene regulation by small RNAs (sRNAs). Commonly sRNAs bind to target messenger RNAs (mRNAs) and modulate the stability and/or translation of the mRNA. To facilitate the interaction between mRNA and sRNA, the Sm-like protein Hfq is needed in case of most trans-encoded sRNAs to overcome limited base pairing. In *Rhodobacter sphaeroides* the expression of several sRNAs is related to (photo-) oxidative stress [1]. One trans encoded sRNA that shows increased expression levels under (photo-) oxidative stress is RSs0680a, which is cotranscribed with 3 homologous sRNAs (RSs0680b-d) and one hypothetical protein (RSP_6037). For stress dependent induction, the RSP_6037/RSs0680a-d operon is controlled by an $RpoH_I/RpoH_{II}$-dependent promoter [2]. We could show that overexpression of RSs0680a-d in *R. sphaeroides* leads to enhanced resistance to oxidative stress. A transcriptome analysis revealed serveral mRNAs with changed abundance in the *R. sphaeroides* RSs0680a-d overexpression strain. Many of these mRNAs show relation to the glutathione (GSH) dependent formaldehyde metabolism. A combination of this transcriptome analysis with different bioinformatic approaches pointed out, that the mRNA of *flhR* (RSP_2591), a transcriptional activator in the GSH dependent formaldehyde metabolism, is a putative target of RSs0680a-d. Interaction of RSs0680a with the *flhr* mRNA could be verified *in vivo* by use of a reporter system for mRNA-sRNA interactions. Moreover we observed increased GSH levels in the RSs0680a-d overexpression strain in comparison to an empty vector control.

This leads us to the conclusion that RSs0680a-d help to increase the free GSH pool under stress conditions by repressing a metabolic pathway that uses high amounts of GSH.

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BRP016

Identification and characterization of toxin-antitoxin systems in *Pseudomonas aeruginosa*

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Type II toxin-antitoxin (TA) protein pairs are encoded by adjacent, co-transcribed genes. The toxins mostly act as mRNases decreasing the global translation rate in bacterial cells or, more rarely, inhibiting DNA replication. The activity of these growth inhibitors is modulated by antitoxins that counteract the inhibitory effect of their cognate toxins by direct protein-protein interaction. Toxins are far more stable than the relative antitoxins, and the latter are rapidly degraded by proteases under unfavourable conditions. Type II TA loci are highly abundant in free-living bacteria, in particular, many pathogenic bacteria contain a high number of predicted TA genes (1). We report about the identification of yet uncharacterized TA systems in the opportunistic human pathogen *Pseudomonas aeruginosa*.
E. coli K-12 TA systems were shown to be required for persistence upon treatment with high doses of antibiotics (2). Persistence is defined as a multidrug tolerance and it is different from resistance since it does not involve genetic changes. We investigated the impact of the newly identified TA loci of *P. aeruginosa* on persistence after antibiotic treatment and find a strong correlation.

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BRP017

Correlation between iron metabolism and oxidative stress response in *Rhodobacter sphaeroides*

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Iron is an essential element for all living organisms. However, since iron potentiates oxygen toxicity by the production of hydroxyl radicals in the Fenton reaction, life in the presence of oxygen requires a strict regulation of iron metabolism. A link between iron metabolism and oxidative stress is well established. A loss of iron regulation causes oxidative stress [1], while oxidative stress affects the expression of several genes involved in iron metabolism [2].
We studied the role of the Irr homologue RSP_3179 in the photosynthetic alpha-proteobacterium *Rhodobacter sphaeroides*. Deletion of Irr (Δ*irr*) had only little effect on growth under iron-deficient conditions, and transcriptome analysis revealed that many genes involved in iron metabolism only showed a slightly lower expression level in the Δ*irr* background. Therefore, we conclude that Irr has no major function in iron regulation in *R. sphaeroides*. However, its deletion resulted in increased resistance to oxidative stress, which correlates with an elevated expression of *katE* for catalase in Δ*irr* compared to the wild type under non-stress conditions [3].
To discriminate between direct effects of iron availability on gene expression and secondary effects that are caused by oxidative stress due to iron limitation, we performed genome-wide transcriptome analyses in the presence or absence of oxygen. These studies revealed that some genes of the iron metabolism as well as some sRNAs respond differently to iron limitation in the presence or absence of oxygen. E. g., the RSs0827 small RNA (sRNA) and the *suf* operon exhibit a strong induction to both iron limitation and oxidative stress under semiaerobic conditions. However, under anaerobic conditions they are not longer induced upon iron depletion. Thus, their induction seems to be mainly dependent on oxidative stress, which in turn is triggered by iron depletion under oxic conditions. This indicates a more indirect function of these genes in iron homeostasis.

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BRP018

The regulatory system of cyanobacteria: an RNA perspective

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The ability for adaptation to vastly different environmental conditions suggests the existence of sophisticated regulatory mechanisms in cyanobacteria. Therefore, regulatory proteins can be expected to interplay with various types of regulatory RNA molecules within the different signal transduction pathways and stress responses. We have experimentally characterized the transcriptomes of the unicellular model organism *Synechocystis* sp. PCC6803 and of the nitrogen-fixing filamentous *Anabaena* sp. PCC7120, identifying more than 3,000 and 10,000 active promoters, respectively. Only about 35% of all promoters drive the expression of protein-coding genes and operons, whereas the remaining 65% transcribe various types of non-coding RNAs (ncRNAs). We show some of these non-coding RNAs fulfill distinct roles in the control of stress responses and the optimization of photosynthesis. Modelling the molecular and functional interactions of cyanobacterial ncRNAs globally suggests an array of distinct molecular mechanisms that need to be integrated into the fabric of the intracellular regulatory network.

BRP019

The alternative sigma factor PP4553 is a negative regulator of antibiotic resistance and biofilm formation in *Pseudomonas putida* KT2440

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Pseudomonas putida, a Gram-negative soil bacterium, is well known for its extreme metabolic versatility, which is at least in parts driven by sophisticated and coordinated regulation of gene expression mediated by a repertoire of transcriptional regulators, in particular the so called sigma factors. Sigma factors are a subunit of prokaryotic RNA polymerases and play a crucial role in transcription initiation by providing promoter recognition specificity. Bacteria generally contain one housekeeping sigma factor and a pool of alternative sigma factors that are activated in response to different and often stressful conditions. The genome of *P. putida* exhibits a striking number of 24 known and putative sigma factors.In order to identify new key regulators, we analysed selected sigma factors for altered antimicrobial resistance and biofilm formation and recognized the alternative sigma factor PP4553 to be involved in these processes in *P. putida* KT2440. We subsequently constructed the knock-out deletion mutant *P. putida* ΔPP4553 and characterised this mutant in more detail. *P. putida* ΔPP4553 exhibited a 2 - 4 fold increase in resistance to antibiotics of various classes. Moreover, the mutant showed enhanced attachment and biofilm formation in comparison to the wild type. We analysed the transcriptome of the ΔPP4553 mutant in comparison to the wild type strain using Illumina RNA sequencing technology resulting in the detection of 35 differentially regulated genes, 19 of which were up- and 16 down-regulated in the mutant. The gene cluster *ttgABC* encoding a RND-type efflux pump was up-regulated in the mutant pointing towards a contribution of this pump in the increased antibiotic resistance. Antibiotic resistance of the mutant was reduced upon addition of a multi-drug efflux pump inhibitor confirming the function of TtgABC as an efflux pump. The direct regulon of PP4553 will be further analysed by combined laboratory and bioinformatic methods.

BRP020

The role of insertion sequence IS*256* in genetic flexibility and development of antibiotic resistance in *Staphylococcus aureus*

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Staphylococcus aureus is a pathogen that causes nosocomial and community-acquired infections. In recent years *S. aureus* has acquired resistance to nearly all antibiotics used in clinical practice. However, exposure to subinhibitory concentrations of antibiotics will not only select for resistant bacteria but may lead to an activation of mutational mechanisms, as for example the SOS response [(1)] or the mobilization of bacterial insertion elements. IS elements form a component of transposons and thus they are involved in the transfer of resistance genes between strains and species. Furthermore, the integration of an IS element into a gene or its promoter may result in an inactivation or overexpression of the affected gene. It has been shown previously that the transposition frequency of a recombinant IS*256* element is activated after the treatment with subinhibitory concentrations of different antibiotics in *S. aureus* HG001. Additionally, the activity of the stress sigma factor B inhibits the transposition of IS*256* by generating an antisense RNA of the transposase using a SigB promoter on the opposite strand of *tnpA* [(2,3)]. We identified the 3' end of the *rsbU* gene, which encodes a positive regulator of sigma factor B, as a hotspot for IS*256* insertion in the clinical isolate *S. aureus* SA137/93G as well as in the laboratory strain *S. aureus* HG001 containing IS*256* on a plasmid. The *rsbU*::IS*256* insertion mutants showed strong hemolysis and displayed a white colony colour as a consequence of inhibited staphyloxanthin biosynthesis. Since SigB cannot be activated in the *rsbU*::IS*256* insertion mutant, the synthesis of *tnpA* antisense RNA from the SigB promoter was repressed. Consequently, we demonstrated that the *rsbU*::IS*256* insertion in strain *S. aureus* HG001 W5 led to a 4-fold activation of IS*256* transposition compared to the *rsbU*-positive control strain. Additionally, we showed that the presence of IS elements like IS*256* promotes the development of vancomycin resistance in *S. aureus* HG001 after serial passaging in the presence of increasing vancomycin concentrations.

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BRP021

Detailed analysis of c-di-GMP mediated regulation of CsgD expression in *Salmonella typhimurium*

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Salmonella typhimurium is capable to form biofilms which contribute to the persistence of the pathogen, mediate resistance to disinfectants and long term desiccation as well as the colonization of the gastrointestinal tract. The secondary messenger cyclic di-GMP promotes biofilm formation by up regulating the expression of CsgD, the major regulator of rdar biofilm formation. C-di-GMP is synthesized by GGDEF domain containing proteins and degraded by EAL domain containing proteins. Among twenty c-di-GMP metabolizing proteins, two GGDEF domain proteins are known to up regulate the CsgD expression and four EAL domain proteins are known to down regulate CsgD expression. Here we identify two novel GGDEF domain proteins required to promote optimal CsgD expression. In addition we show that diguanylate cyclase activity of the GGDEF domain proteins is essential for up regulation of biofilm formation and phosphodiesterase activity of EAL domain proteins for down regulation of biofilm formation in *Salmonella typhimurium*. The c-di-GMP signaling network regulating CsgD expression is highly complex with corresponding di-guanylate cyclases and phosphodiesterases. The contribution of GGDEF/EAL domain protein besides turnover of c-di-GMP is minor. High c-di-GMP levels mediated by the deletion of major phosphodiesterases do not affect translation and function of CsgD suggesting that up regulation of CsgD expression over wild type levels requires mechanisms prior to translation.

CBV001

Towards understanding the planctomy-cetal cell biology

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Planctomycetes are ubiquitous environmentally important bacteria that comprise conspicuous traits usually related to eukaryotes rather than bacteria. For example an intracytoplasmic membrane divides their cytoplasm into multiple compartments while their cell walls lack peptidoglycan. In addition, some *Planctomycetes* can take up proteins employing vesicles in an endocytosis like manner paralleling this eukaryotic hallmark trait. Further more, all planctomycetal species comprise complex life cycles involving a juvenile planktonic swimmer- and an adult sessile stalked stage. Exclusively stalked, sessile cells can divide FtsZ independent through budding. Such traits that are mostly unique among bacteria make the planctomycetal cell biology worth to explore. Until recently, analyzing the molecular basis of such traits was hampered by the lack of genetic tools for *Planctomycetes*. We recently constructed such tools for *P. limnophilus*. In addition, we developed a fluorescent protein tagging approach for *Planctomycetes* to allow determining the spatial localization of proteins of interest. Our proof of principal experiments demonstrates that the expression of translational fusions to GFP is possible in *P. limnophilus* employing either a Tn5 based mutagenesis strategy, or site directed mutagenesis. We further developed a shuttle plasmid capable to replicate in both, *E. coli* and *P. limnophilus* and demonstrated successful GFP expression in trans. In addition to light microscopic analysis we employed cryo-electron tomography to study the cell biology of *P. limnophilus*. If both microscopic approaches are correlated, we expect to unearth the molecular mechanisms that make *Planctomycetes* unique in terms of their cell biology.

CBV002

The actin homolog MreB organizes the bacterial cell membrane

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The morphology of most rod-shaped bacteria is established by the coordinated incorporation of new cell wall material perpendicular to the cell axis (1). An essential component of this machinery is the bacterial actin homolog MreB (2). The prevailing model in which membrane associated MreB-filaments direct the synthesis of new peptidoglycan strands has recently been revised. It turned out that MreB, and the associated cell wall synthetic machinery, move around the cell in a process driven by peptidoglycan synthesis and not by ATP-dependent polymerization of MreB (3-5). Thus, exact molecular function of MreB in this complex remains elusive.
Previously, we have shown that the MreB cytoskeleton of *Bacillus subtilis* is sensitive to changes in the membrane potential, and incubation with ionophores dissipating the membrane potential results in a rapid delocalization of MreB (6). During this work, we noticed that the fluorescence of the cell membrane, when stained with the lipid dye Nile Red, also shows a transformation from a uniform into a clustered fluorescent signal which indicates irregularities in the membrane structure. Analysis of this effect allowed us to determine a novel and conserved activity of MreB in organizing cytoplasmic membrane. By using specific lipid dyes we show that MreB creates fluid domains in the cell membrane of both *B. subtilis* and *E. coli*. The association of fluid lipid domains with MreB results in a complex domain-architecture of the cell membrane and disruption of this domain network leads to an altered distribution of membrane proteins. For two tested *E. coli* proteins LacY and F_1F_o ATP synthase this is also accompanied with reduction of their transport activity. Furthermore, the active movement of MreB and the associated fluid lipid domains is able to generally stimulate the diffusion of membrane proteins. This novel property of MreB has an intriguing resemblance to the role of cortical actin cytoskeleton in organizing the membrane in eukaryotic cells.

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CBV003

The isolation of a 'prokaryotic cell organelle' from an anammox bacterium

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The bacteria capable of anaerobically oxidizing ammonium (anammox) [1] play a major role in the global nitrogen cycle, due to their large contribution to the oceanic nitrogen loss [2], and they are also successfully applied for the removal of ammonium from municipal wastewater. One of their many striking features is their complex internal organization; the anammox cells are divided into three separate compartments [3]. The outermost compartment is called the paryphoplasm and is thought to be dissimilar from the periplasmic space in Gram-negative bacteria, although its function is still unknown. It is separated by an intracytoplasmic membrane from the riboplasm which harbors the RNA and DNA of the cell. The innermost compartment is called the anammoxosome and is hypothesized to be the site of the energy metabolism, analogous to eukaryotic mitochondria [4,5].
The anammoxosome from the anammox bacterium *Kuenenia stuttgartiensis* was isolated by physical and chemical disruption techniques. This resulted in two subcellular fractions which were separated by density gradient centrifugation. The isolation products, i.e. isolated anammoxosomes and cells without paryphoplasm, were imaged using both immunofluorescence microscopy and transmission electron microscopy. In addition proteome as well as lipid analyses were performed. The latter focused on the internal distribution of ladderane lipids which are only found in anammox bacteria and are thought to be enriched in the anammoxosome membrane [6].
Future studies will attempt to demonstrate the catabolic activity and ATP generation of the isolated anammoxosomes.

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CBV004

Stress-induced protein misfolding arrests the *Caulobacter crescentus* cell cycle

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The decision to initiate DNA replication is a critical and highly regulated step in the cell cycle of all organisms. Cells often delay replication initiation in the face of nutrient limitation or stressful conditions, but the underlying mechanisms remain incompletely defined. In bacteria, the replication factor DnaA initiates every new round of DNA replication and hence has to be tightly controlled. Here, we demonstrate that in *Caulobacter crescentus* the stability of DnaA is regulated in response to changes in the global protein folding state. A well-balanced protein homeostasis depends on the activity of molecular chaperones that assist protein folding. We find that the highly conserved DnaK/Hsp70 chaperone system promotes the accumulation of stable DnaA. Depletion of DnaK, or sequestration of the chaperone by unfolded proteins during heat shock, triggers the synthesis of the Lon protease, which can directly degrade DnaA. Unexpectedly, we find that unfolded proteins that accumulate following loss of DnaK also allosterically activate Lon to degrade DnaA. Given that a wide range of stresses can cause the misfolding of proteins, our work reveals a new cellular mechanism for regulating DNA replication and cell cycle progression under adverse growth conditions.

CBV005

Attachment of PHB Granules to the DNA Is Mediated via PhaM in *Ralstonia eutropha*

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Ralstonia eutropha H16 has become the model organism for studying metabolism of poly(3-hydroxybutyrate) (PHB), an important biodegradable biopolymer [1]. We recently identifed a new PHB granule associated protein, PhaM, by interaction with PHB synthase PhaC in a *E. coli*-based two hybrid assay [2]. In this contribution we were able to show expression of PhaM (in dodecamer form) and to demonstrate the interaction between PhaC and PhaM by bimolecular fluorescence complementation (BiFC) in the native strain *R. eutropha*. Evidence is provided for the first time that PhaC in the absence of PHB is not a soluble enzyme but is bound to chromosomal DNA via its interaction with PhaM. Transmission electron micrograph analysis confirmed our BiFC data and showed that PHB granules were always localized in close association with the nucleoid [3]. Overexpression of PhaM resulted in formation of an increased number of small nucleoid-bound PHB granules with raspberry like appearance by TEM. A revised model for PHB granule formation will be proposed.

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CBV006

You are what you talk: Quorum sensing induces individualisation of the algal symbiont *Dinoroseobacter shibae* DFL-12

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In the last decades it became more and more apparent that physiological heterogeneity is a fundamental characteristic of isogenic bacterial populations. It may be induced by stochastic gene expression or multistable gene regulatory networks. *Dinoroseobacter shibae* is a member of the Roseobacter clade abundant in marine environments and is found associated with dinoflagellates.
D. shibae cells show an astonishing heterogeneity with respect to cell shape - ranging from 0.5 μm ovoid rods to filamentous cells more than 10 μm in size - and to cell division. As revealed by time-lapse microscopy, cells divide by binary fission or budding from one or both ends.
However, this phenotypic variability is lost when the quorum sensing (QS) system of *D. shibae* is silenced. QS refers to a form of cell-to-cell communication that involves production, excretion and detection of small diffusible signalling molecules called autoinducers (AI). In Gram-negative bacteria it is mediated through acylated homoserine lactones (AHLs). *D. shibae* utilises a complex communication system involving three AHL synthases (*luxI*1-3). It produces novel AHLs with unsaturated C18 side chains. We constructed aΔ *luxI*1-knock-out strain completely lacking AHL biosynthesis. The QS null mutant cells were uniform in size and cell division and showed a higher growth rate than the wild-type.
Transcriptome analysis revealed that genes responsible for cell cycle control are repressed in $\Delta$the *luxI*1-knock-out strain. In addition flagella biosynthesis and type IV secretion were down-regulated. The wild-type phenotype and gene expression could be restored by genetic complementation as well as through addition of synthetic C18-AHLs. The effectiveness of synthetic AHLs was dependent on the number of double-bonds in the acyl side chain and on the regulated trait, with only the type IV secretion being induced by foreign AHLs. Such graduated sensitivity might enable a modulated response to signals synthesized by other bacteria. We conclude that QS in *D. shibae* rather than coordinating cells in a population, induces phenotypic individualisation. This might be a beneficial strategy to survive in changing environments e.g. during algal blooms when *D. shibae* has to compete with other bacteria for resource utilisation and is subjected to grazing.

Reference: PMID: 19741735

CBV007

Excess membrane synthesis drives a primitive mode of cell proliferation

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The cell wall is a defining structural feature of the bacterial subkingdom. However, most bacteria are capable of mutating into a cell-wall-deficient "L-form" state, requiring remarkable physiological and structural adaptations. L-forms proliferate by an unusual membrane deformation and scission process that is independent of the conserved and normally essential FtsZ based division machinery, and which may provide a model for the replication of primitive cells. We recently developed a tractable system for studying the cell biology and genetics of L-forms in *Bacillus subtilis* [1] and used this system to look for genes required specifically for L-form growth [2]. We found no evidence for involvement of cytoskeletal proteins in L-form proliferation but instead identified the synthesis of certain branched chain fatty acids as being critical for a late step in separation of progeny cells (scission).
Here, we will present the genetic requirements needed to generate a proliferative L-form in *Bacillus subtilis* and show that the key mutational event works by promoting excess membrane synthesis. The results suggest that an increased cell surface area / volume ratio drives the formation of pulsated cell shape change leading to cell division in L-forms. Our findings support models for the early evolution of life in which purely biophysical processes could have supported efficient cell proliferation and provide an extant biological model for studying this problem.

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CBV008

Imaging intact bacterial cells in a life-like state, in three dimensions and to mole-cular resolution

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Groundbreaking insights in Bacterial Cell Biology were facilitated in particular by advances in fluorescence light microscopy (fLM) and electron microscopy (EM). Electron cryotomography (ECT) can elucidate the 3D *in vivo* structure of a unique object at molecular resolution by imaging frozen-hydrated intact cells [1]. One showcase is the bacterial cytoskeleton, which can be directly visualized only by ECT. Today we know that bacterial cytoskeletal proteins polymerize into surprisingly diverse superstructures, which led us to propose a new definition for the bacterial cytoskeleton [2]. The evolution of each superstructure was driven by specific functional requirements. Thus, the *in vivo* superstructure of a respective cytoskeletal element is the key to understand its function. For instance, despite a wealth of available data on the type VI secretion (T6S) system, nobody hypothesized that it involves a cytoplasmic spring-loaded dagger-like superstructure; however, the mechanism was immediately clear by combining our ECT data with insights from live cell fLM [3]. The *in vivo* superstructure can be tremendously helpful to understand cytoskeleton evolution as well. Our finding that bacterial tubulins assemble into five-protofilament bacterial microtubules gave first insight into microtubule evolution [4]. Two more recent projects illustrate the potential of ECT to uncover artifacts of conventional EM. Imaging *Planctomycetes* and *Verrucomicrobia* revealed that the description of a unique compartmentalized cell plan was a misinterpretation of conventional EM data. While we observed exceptional invaginations of the cytoplasmic membrane, no true intracytoplasmic compartment was seen. Further, we found that crescent bodies, which have been described as a developmental stage of chlamydiae, are an artifact of fixation/dehydration. Very recently, we investigated for the first time bacteria inside their host by ECT. We uncovered interactions of chlamydial inclusions with host organelles, and found a novel class of T6S-related structure in amoeba symbionts.

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CBP001

Crystal structure determination and biochemical studies reveal that pesticin, a protein toxin from *Yersinia pestis,* is derived from phage lysozymes

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Yersinia pestis secretes a bacterial protein toxin named pesticin (Pst) that kills related bacteria of the same ecological niche by cleaving the murein (peptidoglycan). In order to get to the target in the periplasm the activity domain (A domain) of Pst (lysozyme) is equipped with a receptor domain (R domain) through which it binds specifically to the FyuA receptor protein at the surface of sensitive cells and a translocation domain (T domain) for translocation across the outer membrane. The R and T domain sequences and crystal structures are unique and reflect the specific import mechanism. In contrasts, the crystal structure of the A domain strongly resembles the crystal structure of phage T4 lysozyme (e gene product) despite of only 13% sequence identity. Replacement of the A domain by T4 lysozyme results in a toxic protein in which T4 lysozyme is carried by the pesticin R and T domains into the periplasm. The protein unfolds to enter the periplasm from outside cells and from the cytoplasm of a deriivative with an added signal peptide. Cross-linking by introduced cystine bridges prevents uptake which is relieved by reduction. Mutations in the predicted active site inactivate Pst and show a similar but not identical reaction center to phage T4 lysozyme.

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CBP002

Interaction of functionalized γ-Fe_2O_3 nanoparticles with influenza A(H1N1)

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Aim of the study: The antimicrobial activity of metal nanoparticles and the widespread use of iron nanoparticles for environmental remediation are explored in recent years. The antiviral activity of γ-Fe_2O_3 nanoparticles was synthesized, characterized and evaluated for its antiviral activity against pandemic influenza A/H1N1 (2009) virus in Madin Darby Canine Kidney (MDCK) cells.
Methods and Results: Effective inhibitory concentration (EC_{50}) of synthesized particles against influenza A/H1N1virus was determined as 20μg/ml. Transmission Electron Microscopic studies (TEM) confirmed that the size of synthesized γ-Fe_2O_3 nanoparticles was 50nm in size, further it explained very well that particle internalization and interact with influenza virus in the MDCK cells. RT-PCR and western blot analyses revealed that, at the 20μg concentration of γ-Fe_2O_3 nanoparticles found to be significantly inhibiting the influenza viral RNA synthesis in MDCK cells. This research focuses on evaluating the interaction of γ-Fe_2O_3 nanoparticles with a pandemic H1N1 influenza virus, to determine if they influence viral replication. Surprisingly exposing the virus to γ-Fe_2O_3 nanoparticles prior to infection actually facilitated virus uptake into the host cells, but the γ-Fe_2O_3-treated virus had a significant reduction in viral RNA synthesis and progeny virus release, which indicates that γ-Fe_2O_3 nanoparticles are capable of inhibiting influenza virus infection *in vitro*.
Conclusions: The γ-Fe_2O_3 nanoparticles inhibiting influenza virus at non-toxic concentrations and effectively inhibit influenza virus replication when administered prior to viral infection. This suggests that the mode of action of viral neutralization by γ-Fe_2O_3 nanoparticles occurs during the initial stages of viral replication.

CBP003

The function of the Na^+-driven flagellum of *Vibrio cholerae* is determined by osmolality and pH

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Vibrio cholerae is motile by its polar flagellum which is driven by a Na^+-conducting motor complex located in the cytoplasmic membrane [1]. The motor consists of a rotor and several stator complexes, each composed of

four PomA and two PomB subunits that provide access of Na^+ to the torque-generating unit of the motor [2, 3]. To characterize the Na^+ pathway formed by the single transmembrane helix of PomB and two transmembrane helices of PomA, we studied the influence of chloride salts (Na^+ or K^+) and pH on the motility of a *pomAB* deletion strain of *V. cholerae* expressing PomB together with PomA encoded on a plasmid. Motility decreased when the proton concentration was lowered, but increased when the chaotrope chloride was added. These results are not in accord with Na^+ and H^+ competing for binding to the conserved D23 in PomB. Cells expressing the S26A/T or D42N variants of PomB lost motility at low [Na^+] at pH 7 and pH 9 but regained some motility in the presence of 170 mM chloride at pH 7. Both PomA and PomB were modified with radioactively labeled *N,N´*-dicyclohexylcarbodiimide (^{14}C-DCCD), indicating the presence of carboxylic residues in hydrophobic environments of the proteins in their protonated state. Na^+ did not protect PomA and PomB from modification with ^{14}C-DCCD. Our study shows that both osmolality and pH have a significant influence on the function of the Na^+-dependent flagellum from *V. cholerae*. We propose that D23, S26 and D42 of PomB are part of an ion conducting pathway formed by the PomAB stator complex.

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CBP004

The cell wall channel of the mycolic acid containing actinomycete *Dietzia maris* and its biophysical properties

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The genus *Dietzia* comprises bacteria from the soil and there is a possibility that they play a significant role in waste water treatment due to their presence in activated sludge (1). *Dietzia* belongs to the mycolata which have beside their thick peptidoglycan layer large amounts of lipids in form of mycolic acids in their cell wall (2). The mycolic acid layer represents a permeability barrier on the surface of mycolata and channels similar to porins of gram-negative bacteria have been identified to exist in the cell wall of these bacteria for the passage of hydrophilic compounds (3-6). To identify the protein responsible for the formation of the cell wall channel in *Dietzia maris*, the cells were treated with a number of different buffers to remove most of the soluble cell wall components. Final purification of the channel-forming protein was achieved by excision of protein bands from tricine-containing preparative SDS-PAGE and extraction of the bands with 1% Genapol, 10 mM Tris-HCl, pH 8 followed by analysis of the bands for channel formation in lipid bilayers. Using this method highest pore-forming activity was found for a 120 kDa band of SDS-PAGE. The eluted protein appeared to be essentially pure. Channel-formation by the 120 kDa cell wall channel of *Dietzia maris* in lipid bilayer membranes was studied in detail. Channel-formation was very frequent and the lifetime of the channels was very long typical for that of cell wall channels of gram-positive bacteria. The channels had a high single-channel conductance of 5 nS in 1M KCl. Further information on the structure of the channel was obtained from single-channel experiments in different salts and different KCl-concentration. Additional information was also obtained from ion selectivity measurements in presence of KCl, LiCl and KCH_3COO gradients. Taken together the results indicated a preferential movement of cations through the cell wall channel. Otherwise the channel is wide and water-filled. Voltage-dependence is another interesting feature of the cell wall channel of *Dietzia maris*. These measurements were performed using membrane potentials in a range between−100 and +1 00 mV and demonstrated that the channel is highly voltage dependent in an asymmetric manner. The pure 120 kDa protein will be subjected to amino acid sequence analysis using Edman-degradation for identification of the gene responsible for channel-forming activity.

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CBP005

Identification and characterization of channel-forming proteins in the cell walls of *Corynebacterium jeikeium* and *Corynebacterium urealyticum*

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The suborder *Corynebacterineae* shares with the mycolata the property of having an unusual cell envelope composition and architecture [2, 4]. The outer membrane of mycolata represents a permeability barrier similar to that of Gram-negative bacteria and uses specialized pore-forming proteins, called porins, to facilitate the passage of water-soluble solutes [1, 5]. Many species within this diverse group of mycolic acid containing actinomycetes are known either because of their medical or biotechnological relevance. The emergence of drug resistance in the clinical environment has been a constant threat over the past decades. Therefore, design of new antibiotics based on the knowledge of porin properties could be helpful to control pathogenic microorganisms that have a natural resistance against antibiotics such as *Corynebacterium jeikeium* and *Corynebacterium urealyticum*. These two pathogenic *Corynebacterium* species belong also to the *Corynebacteriaceae* family [6]. The cell wall of *C. jeikeium* contains a 40AS channel-forming protein, CjPorA, coded by *jk0268*. CjPorA protein was expressed in *C. glutamicum* Δ AH pXMJ19-ct-His. Biophysical characterization of the purified CjPorA demonstrated that the protein formed channels with a conductance of about 1.5 nS in 1M KCl under low voltage conditions (20 mV). The study of CjPorA will be continued by extending its investigation to the genetic engineering of the protein.*C. urealyticum* represents another pathogenic member of the genus *Corynebacterium*. As the genome of *C. urealyticum DSM 7109* strain (taxid:504474) has recently been sequenced (8), we searched for additional information of unknown cell wall channels, by using BLAST search within the chromosome for the presence of porA- and porH-like porin genes in *C. urealyticum*. This search suggested that the *porH* and *porA* genes could be localized in a chromosomal region that is similar to that of the other species investigated so far. To answer this question corresponding genes within the genomic sequence of C. *urealyticum* were cloned into pGEM-T EASY vector. Likewise the N-terminal truncations were generated which have a N-terminal GST fusion protein for expression in *E. coli BL21(DE3)omp8*.
The next step could be the final identification of the pore and its channel-forming properties in lipid bilayer experiments.

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CBP006

The dynamical cytoskeleton regulates morphogenesis in rod-like bacteria

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Bacteria were long regarded as unstructured bags of freely diffusing proteins and DNA. Contrary to this view, bacterial cells are now known to display intricate sub-cellular localization and dynamics of their constituents, which is required for a variety of processes including cellular morphogenesis. Biophysically, one mechanism for achieving sub-cellular order relies on the bacterial cytoskeleton, which consists of polymeric proteins that are often distant homologs of the cytoskeletal proteins found in eukaryotic cells. While many of these proteins have been identified and structurally characterized in the recent years, their dynamics are poorly understood.
Here we report a quantitative study of the dynamics of the Escherichia coli actin homolog MreB, which is essential for the maintenance of rod-like cell shape in bacteria (1). We found that MreB rotates around the long axis of the cell in a persistent manner and that this rotation depends on the assembly of the peptidoglycan cell wall. Biophysical modeling suggests that MreB and cell-wall synthesis are physically coupled. Thus, the MreB motion observed constitutes a reporter of the local insertion of cell-wall material. In agreement with recent experiments on macroscopic twisting of the cell

envelope during growth (2) we find that peptidoglycan is deposited in the cell wall in a helical manner. The cell wall in turn ultimately determines bacterial cell shape. Semi-atomistic computational simulations suggest that one function of MreB is to ensure a uniform distribution of new peptidoglycan insertion sites, a necessary condition to maintain rod shape during growth. Based on the same computational framework we hypothesize that MreB governs bacterial cell shape in a non-trivial manner.
Our findings both broaden the view of cytoskeletal motors and deepen our understanding of the physical basis of bacterial morphogenesis.

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CBP007

Substrate availability guides peptidoglycan synthesis

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Peptidoglycan (PG) is the main component of the bacterial cell wall giving shape to almost all bacteria. PG synthesis is carried out by a family of Penicillin Binding Proteins (PBPs) that incorporate the PG precursor LipidII into a growing PG network. PBPs interact with other proteins such as the actin homologue MreB to form dynamic, membrane-associated polymeric structures that are thought to guide the localization of PBPs and hence cell wall synthesis. Recently, cell wall elongation was shown to be required for the dynamics of MreB, instead of the other way around. This begs the question how PBP localization is organized. Substrate availability is an alternative model for PBP localization.
Here we present a test of the substrate availability model in the MreB containing rod-shaped bacterium *Bacillus subtilis*. We monitored the localization of GFP-PBPs after active delocalization of the PBP substrate LipidII. Delocalization of LipidII resulted in delocalization of PBPs that drive PG synthesis during elongation. We have extended our analysis to the oval-shaped *Streptococcus pneumoniae* which lacks MreB, and show that LipidII delocalization leads to PBP delocalization in this organism as well. Our results show that LipidII localization, and thus substrate availability, is critical for the localization of PBPs that were initially thought to be guided by the cytoskeleton.

CBP008

Functional characterisation of the DivIVA-homologue GpsB in *Listeria monocytogenes*

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GpsB is a DivIVA homologue which is present in different *Firmicutes* such as *Bacillus subtilis, Staphylococcus aureus* and *Listeria monocytogenes*. A *gpsB* deletion in *B. subtilis* leads to a higher salt sensitivity and a defect in cell division and cell elongation as *gpsB* cells of *B. subtilis* revealed an unusual bulging phenotype (1). In contrast, pilot experiments in *L. monocytogenes* showed that *gpsB* apparently is essential as we only could remove the chromosomal *gpsB* gene in the presence of a second ectopically expressed copy of *gpsB*. This observation prompted us to investigate the function of GpsB in more detail and hence we analysed possible interaction partners in the bacterial two hybrid (B2H) assay. These experiments demonstrated that four of the five high molecular weight penicillin binding proteins (HMW-PBP) of *L. monocytogenes* interact with GpsB. Moreover, GpsB uses its N-terminal lipid binding domain to interact with these PBPs as we concluded from the analysis of GpsB truncation variants in B2H experiments. The subcellular localization of all listerial HMW-PBPs and of GpsB was studied by fluorescence microscopy and demonstrated that GpsB and the HMW-PBPs are located at similar areas in the cell and especially accumulated at the division sites, suggesting that GpsB might serve as a topological determinant for PBP localisation. We want to describe a *gpsB* phenotype for *L. monocytogenes*, and are therefore currently constructing *gpsB* depletion strains. Current results of this ongoing project will be presented on this poster.

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CBP009

Analysis of the apical cell growth machinery from *Corynebacterium gluta-micum*

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Corynebacterium glutamicum is a Gram-positive soil bacterium with high industrial importance. Furthermore, it serves as a non-pathogenic model organism for related pathogens causing tuberculosis, diphtheria and leprosy. Like its pathogenic relatives, *C. glutamicum* lacks several conserved cell division and shape determining proteins such as the actin homologue MreB, the nucleoid occlusion system Noc and the division site selecting Min system, which all play important roles in the model organisms *E. coli* or *B. subtilis* for instance. Instead, morphology and polar elongation is ensured by a protein complex composed of the polar determinant DivIVA and several penicillin-binding proteins (PBPs). To date, only little is known about the spatial and temporal regulation of the apical cell growth machinery, which has been proposed as new target for antibiotic (AB) intervention.
We recently showed that DivIVA directly interacts with the Par system, thereby acting as a polar tethering factor in chromosome segregation (Donovan *et al*., 2012). Furthermore, we now provide evidence that DivIVA interacts with the Lipid II flippase RodA, thereby spatially regulating apical cell growth. Depletion of *divIVA* as well as deletion of *rodA* results in a coccoid morphology. In addition a Δ*rodA* strain had a strong growth defect and increased sensitivity to several ABs. A complementation strain allowed subcellular localization of RodA-GFP at the cell poles. Using our established synthetic *in vivo* system, where *E. coli* cells are used as expression vessels for protein-protein interaction candidates, we provide evidence that DivIVA-RodA interaction recruits RodA to the cell poles. A heterologous FRET system with DivIVA-YFP and RodA-CFP confirmed this interaction. To verify the specificity of this interaction, we included the second Lipid II flippase FtsW in our *in vivo* system. However, an interaction of DivIVA and FtsW, which is part of the divisome and localizes exclusively at midcell in *C. glutamicum,* could not be observed. To our surprise, time-lapse microscopy revealed sustained polar growth after *rodA* deletion, implicating a lateral Lipid II movement from midcell to the polar PBPs. This hypothesis could be confirmed with a Vancomycin-FL staining and microscopy analysis.

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CBP010

Molecular basis of symbiosis in motile phototrophic consortia

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Motile phototrophic consortia are highly regular mulitcellular associations in which a flagellated colourless Betaproteobacterium is surrounded by numerous cells of green sulfur bacteria, the so called epibionts. Cell division of the symbiots is highly coordinated and a rapid signal transfer between the epibionts and the central bacterium has been demonstrated. 16S rRNA analysis of epibionts from freshwater lakes and ponds around the world revealed unique 16S rRNA sequence types which have not been found in a free-living state and are not monophyletic. In addition, the genome of *Chlorobium chlorochromatii,* the epibiont of the only consortium which is in culture ("*Chlorochromatium aggregatum*") was compared with genomes of free-living relatives which revealed the presence of unique open reading frames. In order to elucidate if these genetic modules are present in the epibionts of other phototrophic consortia and if the epibionts have coevolved with their particular central partner bacterium, consortia were sampled from Dagow Lake (Brandenburg, Germany), isolated through cell sorting and their genomes amplified. To study the molecular basis of symbiosis four constitutively transcribed putative symbiosis genes (Cag_1919, 0616, 0614, 1920) were analysed in detail. Cag_1919 contains a RTX domain which is typically found in Gram-negative pathogenic bacteria. Cag_0614 and Cag_0616 represent the largest open reading frames in the prokaryotic world known to date with length of 110418 and 61938 bp, respectively. Interestingly, while expressing Cag_1919 and 1920 heterologously in *E. coli,* the strains formed extremely long, filamented or branched cells. To facilitate the localization of the proteins in *Chl. chlorochromatii,* in the free-living and symbiotic state, the resulting recombinant proteins were used to produce antibodies for immunogold labelling and fluorescence microscopy. The intracellular localization of symbiosis factors and the analysis of their

evolutionary context provide novel insights into the molecular basis of symbiosis.

CBP011

Hopping and stumbling into a novel gene related to DivIVA function in *Listeria monocytogenes*

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DivIVA is a cell division protein found to be well conserved in various Gram-positive bacteria. In some, the *divIVA* gene plays an indispensable role in cell viability, while in other species, a heavily perturbed morphology results from its deletion. The diverse morphogenetic functions of its orthologues in different bacterial species are postulated to stem from its ability to bind to different interaction partners (1). One of its putative interaction partners was hypothesized to be the accessory secretion ATPase, SecA2 (2). SecA2 allows for the translocation of virulence related autolysins such as p60 and MurA, contributing to the full virulence of *L. monocytogenes*. Bacterial two hybrid assays and pull down experiments were used to check for any interactions between DivIVA, SecA2, p60 and MurA. However, these experiments hinted to the presence of other intermediary interaction partners due to the absence of any observable direct interactions between DivIVA and these proteins. As an alternative approach for the identification of such putative interaction partners, transposon mutagenesis was employed to randomly perturb the genome, followed by screening for clones bearing phenotypic similarity to both *divIVA* and *secA2* deletion mutants. This strategy helped us identify genes that play a role in SecA2-dependent protein secretion, cell division and virulence pathways of *L. monocytogenes* and led to the identification of a previously unknown gene which seems to be conserved in several *Bacilli*.
Current progress of these experiments would be presented on this poster.

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CBP012

Characterization of epigenetic systems in the regulation of DNA replication, repair and segregation in *Escherichia coli* and *Vibrio cholerae* by methods of single molecule analysis and synthetic biology

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Escherichia coli and *Vibrio cholerae* are closely related and in both organisms the GATC methylation by Dam-methyltransferase is crucial. *V. cholerae* in comparison to *E. coli* has two chromosomes and is therefore an ideal model for studying the organization of the chromosomes in a simple multi-chromosome system.
There are three different states of the GATC site which are fully-methylated, hemi-methylated and un-methylated. The full-methylation is the most common condition and hemi-methylation is limited to a frame behind the replication fork and to the origin of replication. Only a small percentage of GATC sites remain un-methylated. The methylation state plays an important role, since proteins bind specifically to hemi-methylated sites. SeqA for example binds hemi-methylated GATC sites and blocks remethylation by Dam. This is particularly important for the reinitiation of DNA replication, as the binding of the initiator protein DnaA is blocked by SeqA. Another protein that specifically binds to hemi-methylated GATC sites is MutH. This protein is involved in DNA mismatch repair (MMR) by distinguishing the parental and newly synthesized DNA strand. After activation of MMR, MutH specifically nicks the newly synthesized strand and introduces DNA degradation.
One of our aims is to generate a genome-wide mapping of methylated GATC sites of *E. coli* and *V. cholerae* on single molecule level by SMRT sequencing. In further experiments we specifically enrich the origins of replication and hemi-methylated DNA of *E. coli* and *V. cholerae* to gain deeper insight into the mode of re-methylation by Dam. In a second approach we try to explore the connection between MMR and the SeqA protein by methods of synthetic biology. For the experimental setup we want to develop and construct synthetic chromosomes with a highly organized structure to analyze chromosome maintenance.

CBP013

New single cell methods to analyze DNA-replication in bacteria with natural or synthetic secondary chromosomes

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DNA replication is an essential process during the cell cycle. In *Escherichia coli*, which harbors it's genetic information on one circular chromosome, the process of DNA replication has been studied extensively. In contrary, for *Vibrio cholerae,* which is a model organism for multi-chromosome bacteria, the mechanism that regulates DNA replication of both chromosomes (ChrI 2,96 Mb and ChrII 1,07 Mb) is poorly understood. Controversial results have been published regarding the statement that chromosomes initiate their replication synchronously. The contradiction comes in part from a lack of suitable methods. Therefore we would like to generate new tools to investigate on single cell level whether the ChrII replication starts or ends in synchrony with ChrI replication. The following methods will be established in *E. coli* and then be transferred to *V. cholerae* to address the biological question.
A first new approach to determine DNA replication in single cells is BiFCROS (Bimolecular Fluorescence Complementation Repressor-Operator System). To analyze replication patterns based on the copy numbers of chromosomal loci, a LacO/TetO array is inserted next to the locus of interest. The corresponding repressor proteins are fused to either the N- or C-terminus of a split fluorescence protein. Binding of the repressors to the operators reconstitutes the fluorescence protein. Fluorescence signal can be measured by flow cytometry and should be proportional to the copy number.
The second tool to address this question is called eFork-counting (EdU-labeled replication fork-counting). With the help of this method, it is possible to mark newly replicated DNA and therefore replication forks. eFork-counting is based on a thymidine analogon EdU that incorporates into the new DNA strand. In a cyclic addition reaction an azide, which is labeled with a fluorochrome is bound to EdU. Fluorescence signals can be quantified by flow cytometry. Thus, it's possible to determine the number of replication forks, showing if only one or both chromosomes are replicated at a specific point of the cell cycle.
These tools are fundamental to investigate replication patterns for natural occurring multi-chromosome bacteria as *V. cholerae*. However, they will also help to reach our mid-term goal, the implementation of synthetic, secondary chromosomes.

CBP014

Towards a synthetic, secondary chromosome in *Escherichia coli*: Characterization of DNA replication components of the two *Vibrio cholerae* chromosomes

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Every cell reveals systems to replicate, segregate and organize DNA. In order to study this chromosome maintenance we plan to establish a synthetic, secondary chromosome in *Escherichia coli*. For this purpose it is necessary to use a replicon, which does not behave like a plasmid but like a chromosome. The synthetic chromosome will be based on the secondary chromosome (ChrII) of *Vibrio cholerae*. The first step towards the synthetic chromosome is to understand the natural chromosome functionality in *V. cholerae* and identify all factors involved.
Possibly, there exist more than the known proteins participating in *V. cholerae* replication. Therefore the proteins bound at the replication origin and at the replication fork will be analyzed using three related methods. All of them are based on the crosslinking of proteins to DNA in vivo followed by different approaches of purification. First, we would like to apply "Proteomics of isolated chromatin segments" (PICh) in order to determine the proteins at the origins. Within this method the origin-DNA is fished with an origin complementary probe. The origin-bound proteins are eluted and identified by mass spectrometry. The proteins at the replication fork will be analyzed by "isolation of proteins on nascent DNA" (iPOND). Thereby the new DNA is labeled with a thymidine analog EdU. This compound can react with a biotin azide in a chemical click reaction and can subsequently be affinity purified. The further protein isolation and analysis is similar to PICh. The third approach is mChIP, where crosslinked DNA with bound replication proteins as DnaA or SeqA is purified with specific antibodies. Co-purified proteins are then again be analyzed by mass spectrometry. Application of this comprehensive set of methods will give a complete overview of factors involved in the replication of the two *V. cholerae*

chromosomes and lay the fundament for a *V. cholerae* based synthetic, secondary chromosome in *E. coli*.

CBP015

Characterization of the S-layer of the anammox bacterium *Kuenenia stutt-gartiensis*

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"Candidatus Kuenenia stuttgartiensis" is an anaerobic ammonium oxidizing (anammox) bacterium belonging to the order of Brocadiales in the phylum of the *Planctomycetes*. Anammox bacteria are important in nature where they contribute significantly to oceanic nitrogen loss and are applied in wastewater treatment for the removal of ammonium. The cell biology of anammox bacteria is extraordinary; the cells are divided into three membrane-bounded compartments. In addition, the cell wall of *K. stuttgartiensis* does not classify as a typical bacterial cell wall, since it has been hypothesized to lack both peptidoglycan and possibly also an outer membrane typical of Gram-negative bacteria. The question thus arises what the anammox cell wall is composed of and how the structural integrity of the cells is maintained. To answer these questions, the cell wall of *K. stuttgartiensis* was studied via freeze etching experiments. Electron micrographs of these experiments showed the presence of a hexagonal surface layer (S-layer) in the majority of *K. stuttgartiensis* cells. S-layers are crystalline two-dimensional arrays of proteinaceous subunits that make up the outermost layer of many bacterial cell envelopes. Enrichment of the S-layer of *K. stuttgartiensis* cells has indicated a putative S-layer protein with an apparent molecular mass of about 230 kDa. Since S-layers have been previously found to have a shape determining function in some bacteria, it is hypothesized that the S-layer could aid in providing structural integrity to the *K. stuttgartiensis* cell. Currently the putative S-layer protein is purified and characterized and immunogold localization will be used to verify the localization of the putative S-layer protein to the actual surface layer. Ultimately, biochemical analysis and proteomics studies should reveal the chemical composition of the anammox cell wall.

CBP016

A screen for novel polar constituents in the cholera pathogen

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The subcellular organization of cell poles is crucial for many bacterial processes, and a number of proteins require polar localization for their function and localization. However, our knowledge about the mechanisms by which proteins are localized and maintained at the cell poles has remained limited. Here, we investigate cell polarity in *Vibrio cholerae*, the gram-negative pathogen that causes the life-threatening diarrheal disease cholera. We have identified a novel polarity factor, HubP, that is required for robust polar localization of the protein machineries that facilitate chromosome segregation and chemotaxis [1]. Notably, HubP has no similarity to previously identified polar markers or determinants. To elucidate the processes that enable HubP to label the cell pole, we used a synthetic lethal screen to isolate proteins that become essential in absence of HubP. This screen identified a putative cell wall hydrolase, PodA. Further analysis revealed that a mutant strain lacking both PodA and its paralog, PodB, exhibits strong polarity defects and aberrant polar morphology, suggesting that PodAB are required to establish proper cell polarity.

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CBP017

Phosphorylated StkP induces the CiaR/CiaH two-component system in *Streptococcus pneumoniae*: Evidence for a feedback system driving pneumococcal cell-cycle progression

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Streptococcus pneumoniae is an oval shaped Gram positive human pathogen. This oval shape is most likely obtained by the alternation of peripheral cell wall elongation and septal cell wall synthesis. We and others recently showed that the eukaryotic-type serine-threonine kinase StkP localizes to the division site and plays an important role in regulating cell division and cell shape (1,2). How StkP exactly drives the pneumococcal cell cycle remains largely elusive. To examine the physiological response of cells to the active form of StkP, StkP~P, we constructed a strain that allows induction of StkP in absence of its cognate phosphatase PhpP. This leads to the accumulation of StkP~P due to the lack of dephosphorylation by PhpP. Cells producing StkP~P are significantly shorter than wild-type cells, indicating that phosphorylation by StkP induces cell division. Interestingly, western blotting demonstrated an increased turnover of StkP under hyperphosphorylation conditions, suggesting the specific induction of a protease.To test this hypothesis, we performed a DNA-microarray experiment to investigate the global transcriptional changes in response to StkP~P accumulation. Strikingly, hyperphosphorylated cells showed a specific upregulation of the CiaR/CiaH two-component system which is known to be activated by a wide range of cellular stresses (3). As part of this regulon, a gene encoding for the extracellular serine protease HtrA was significantly upregulated upon StkP~P induction. Whether StkP~P is a direct target of HtrA is currently under our investigation. We speculate that StkP, through CiaR/H and HtrA-mediated proteolysis, sets up a finely controlled feedback-system which controls cell-cycle progression in *S. pneumoniae*.

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CBP018

Monitoring of population dynamics of *Corynebacterium glutamicum* by multi-parameter flow cytometry

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Several recent studies revealed that microbial populations may exhibit significant phenotypic heterogeneity at the single cell level. Conventional methods for the analytical monitoring of bioprocesses are, however, still dominated by bulk measurements. In the present study we used flow cytometry to study population dynamics of the Gram-positive amino acid producer *Corynebacterium glutamicum* with regard to cell size, DNA pattern, membrane potential and membrane integrity. Already the analysis of simple scattering properties (forward scatter and side scatter) provided insights into the growth activity of *C. glutamicum* in different growth phases on standard complex and minimal media. Our data confirmed previous studies which described that stationary *C. glutamicum* cells exhibit a smaller cell size than cells of the logarithmic growth phase. Furthermore, we used 4',6-diamidino-2-phenylindole (DAPI) staining for the analysis of the DNA content of single *C. glutamicum* cells. The recorded DNA patterns were shown to be indicative for the particular growth phase and provided insights into proliferation and cell cycle activities of this species. As expected, DAPI patterns indicated uncoupled DNA synthesis in the logarithmic growth phase, whereas cells arrested in a pre-division phase under limiting growth conditions. In further experiments we applied different staining procedures to study membrane potential and integrity of *C. glutamicum* during growth on CGXII minimal medium. Especially in early log and stationary phase cells we observed a considerably high number (approx. 1-5%) of cells with a decreased membrane potential. This subpopulation almost vanished in the exponential growth phase. A similar pattern was also observed when *C.*

glutamicum populations were analyzed with respect to membrane integrity by staining with the fluorescent nucleic acid dyes Syto 9 and propidium iodide (PI). Altogether, our data highlight the potential of flow cytometry as rapid and convenient tool for the online monitoring of bioprocesses and for the multiparameter analysis of *C. glutamicum* populations.

CBP019

Investigation of proteins involved in *Sinorhizobium meliloti* replication initiation and cell cycle control

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Sinorhizobium meliloti is a Gram negative α-proteobacterium capable of entering nitrogen fixing endosymbiosis with *Medicago* host plants. Final steps in the establishment of this symbiosis involve deregulation of the bacterial cell cycle resulting in polyploid enlarged bacteroids. Recent studies in α-proteobacterial cell cycle model *Caulobacter crescentus* imply regulatory inactivation of DnaA (RIDA) is the main mechanism ensuring that chromosomal replication starts only once per cell cycle (Collier and Shapiro 2009; Fernandez-Fernandez et al. 2011). One approach is to classify α-proteobacterial cell cycle regulatory circuits into two modules with DnaA governing replication periodicity and CtrA controlling asymmetric fates of daughter cells (Jonas et al. 2011). To understand the *S. meliloti* bacteroid differentiation process, data acquisition of temporal-spatial dynamics of *S. meliloti* main cell cycle players is essential.
We use time lapse fluorescent microscopy to observe fluorescent translational fusions to key proteins of *S. meliloti* replication initiation, cell cycle progression and cell division modules. New insights into regulation of cell cycle control in the free living state and deregulation of cell cycle protein localization patterns when diffentiating into bacteroids in the symbiotic state will be presented.

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CBP020

Cloning, transfer and expression of large magnetosome operons from the magnetotactic bacterium *Magneto-spirillum gryphiswaldense*

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The alphaproteobacterium *Magnetospirillum gryphiswaldense* produces intracellular organelles, the magnetosomes, which consist of magnetite crystals enveloped by a magnetosome membrane. The uniform sizes and unique magnetic properties make the particles highly attractive for biotechnological and biomedical applications, which, however require methods for genetic engineering and manipulation of the pathway. The *mamAB*, *mamGFDC*, *mms6* and *mamXY* operons located on a 115 kb magnetosome island (MAI), were implicated in the synthesis of properly sized and shaped magnetite crystals. However, cloning, transfer and functional expression of the large clusters encoding magnetosome synthesis has proven difficult. To assay successful transfer of functional genomic fragments, we attempted to complement mutants harboring large deletions. Whereas complementation of deletions up to 5 kb *(ΔmamGFDC, ΔmamXY)* resulted in stable *in trans* expression and reconstitution of wildtype phenotypes, we failed to complement large deletions due to instable expression of large plasmid-born fragments. For complementation of a *mamAB* operon deletion lacking 17 kb, we used ET-based recombinogenic cloning to construct transposon vectors comprising the entire *mamAB* operon. Random genomic transposition by conjugational transfer resulted in restoration of magnetite biomineralization in a non-magnetic *mamAB* mutant. In addition, we used triple recombination to piece together a large (32.5 kb) transferrable cassette harboring all 4 magnetosome operons (30 genes). Cloning and functional expression of the entire gene set controlling magnetosome synthesis will also be useful for future synthetic biology approaches as well as expression in different host organisms.

CBP021

Evidence for a Metabolic Link between PHB and Polyphosphate Metabolism in *Ralstonia eutropha*

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Ralstonia eutropha is famous for its ability to accumulate large amounts of poly(3-hydroxybutyrate) (PHB). Notably, *R. eutropha* can also accumulate polyphosphate (PP). In this study, we performed a bacterial adenylate cyclase-based two-hybrid screening approach to identify potential interaction partners of PHB granule associated proteins. When we used phasin PhaP2, PhaP3 or PHB depolymerase PhaZa1 as bait proteins we identified internal parts of the same prey protein as interaction peptides with all three different bait proteins. The identified prey protein is annotated as hypothetical protein with a predicted function in phosphate metabolism and was designed as PhaX. Subcellular localization of the *phaX* gene product was performed using a variant of GFP. Additionally, a chromosomal Δ*phaX* mutant was constructed and investigated for its ability to form PHB and PP. Mutant cells produced much more PP granules than the wild type as revealed by staining of the cells with DAPI. PHB accumulation in the deletion mutant was not affected. However, reutilization of PHB in the stationary phase was impaired in comparison to the wild type which degraded PHB completely. Also the cell morphology and cell viability was affected by the deletion of *phaX*. Results of a double mutant with deleted PHB synthase gene *(ΔphaX, ΔphaC)* will be also shown. Our results indicate that PHB metabolism is linked to phosphate/PP metabolism. Images showing formation of PHB and PP in wild type and mutant cells will be presented.

CBP022

Flotillins functionally organize the bacterial membrane

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The theory of lipid rafts suggests lateral heterogeneity in plasmamembranes [1]. Contrary to previous assumptions recent studies could demonstrate that lipid rafts are not restricted to eukaryotes but are also present in bacteria [2,3]. A subset of proteins that are routinely used as lipid raft markers are flotillins. Previously we could show that the *B. subtilis* flotillin homologue YuaG is expressed in stationary phase and creates highly dynamic foci at the plasmamembrane [2]. Furthermore, deletion of YuaG leads to disruption of several distinct signaling cascades [2,3]. Here, we could identify interactions partners of YuaG by fusing YuaG to the SNAP-tag and subsequent pull down assays. Among the interacting proteins of YuaG were several components of the secretion machinery. To validate these interactions microscopic and biochemical assays were performed demonstrating that flotillins and the secretion machinery are indeed functionally linked. Parts of the secretion machinery co-localize with YuaG and a loss of flotillins quantitatively decreased secretion efficiency in *B. subtilis*. To further investigate the molecular function of flotillins, YuaG was heterologously expressed and purified. It could be demonstrated that YuaG forms highly dynamic oligomeric structures in mega Dalton size *in vitro*. To elucidate the influence of these oligomers on the bacterial plasma membrane we utilized the anisotropic dye LAURDAN that intercalates into the membrane and exhibits a fluorescence shift upon polarity changes of the environment and thereby monitoring the condensation of the membrane. Using LAURDAN we could show that deletion of flotillins lead to coalescence of distinct domains in *B. subtilis in vivo*. These data provide evidence that flotillins function in membrane domain separation, ensuring membrane heterogeneity.

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CBP023

Towards understanding the molecular mechanism of FtsZ independent cell division in *Planctomycetes*

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Planctomycetes are ubiquitous environmentally important bacteria that comprise conspicuous traits usually related to eukaryotes rather than bacteria. For example an intracytoplasmic membrane divides their cytoplasm into multiple compartments while their cell walls lack

peptidoglycan. In addition, some *Planctomycetes* can take up proteins employing vesicles in an endocytosis like manner paralleling this eukaryotic hallmark trait. Most strikingly, all planctomycetal species comprise complex life cycles involving a juvenile planktonic swimmer- and an adult sessile stalked stage. We found in contrast to previous observations that only sessile *P. limnophilus* cells can divide employing an FtsZ independent enigmatic cell division mechanism. While all *Planctomycetes* divide asymmetrically through budding, only *P. limnophilus* is genetically accessible and thus a suitable model organism to study the planctomycetal cell division. To do so, we first used bioinformatics with a focus on comparative genomics to identify proteins putatively involved in *P. limnophilus* cell division. While the bacterial cell division was thought to rely always on FtsZ, Archaea comprise in addition two FtsZ independent division logics. However, our bioinformatic analysis revealed that *Planctomycetes* most likely posses a fourth, yet unknown mechanism of cell division. Thus, we distilled the planctomyctal core genome and subtracted genes of model organisms such as *E. coli* or *B. subtilis*. Second, we analyzed the gene content and synteny of bacterial cell division related genes other than FtsZ and combined both methods using a "guilt-by-association" approach with a focus on our model organism *P. limnophilus*. We further analyzed putative operons for polycistronic expression employing RT-PCR and developed a translational fusion strategy that allow the visualization of proteins putatively involved in the planctomycetal cell division using time-lapse experiments. Taken together we present a reasonable number of candidate proteins putatively involved in a fourth mechanism of cell division, unknown among Bacteria and Archaea thus far.

CBP024

Spore wall assembly in *Streptomyces coelicolor*

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The Mre-proteins of rod-shaped bacteria form a peptidoglycan (PG) synthesizing complex at the lateral wall to ensure elongation growth. Although mycelial *Streptomyces coelicolor* grows by apical tip extension which does not involve lateral cell wall synthesis, it contains three *mreB*-like genes and a complete *mreB* cluster comprising mreBCD, *pbp2* and *sfr* (*rodA*). Mutant analysis demonstrated that the *mre*-genes were not required for vegetative growth but affected sporulation. Mutant spores suffered from a defective spore wall rendering the spores sensitive to high osmolarity, moderate heat and to cell wall damage by lysozyme or vancomycin[1,2]. Study of protein-protein interactions by a bacterial two-hybrid analysis revealed a similar interaction pattern as reported for the lateral wall synthesizing complex suggesting that the *Streptomyces* spore wall is synthesized by a multi-protein complex which resembles the lateral wall synthesizing complex of rod-shaped bacteria[2]. Screening of a genomic library identified several additional interaction partners as novel components of this complex. Interaction of MreC, MreD, PBP2 and Sfr with SCO2578 and SCO2584 suggested an involvement of wall teichoic acid biosynthetic enzymes. Knock out experiments confirmed this assumed role in spore wall synthesis. Mutants lacking the putative wta genes *sco2584* or *sco2997* showed a similar spore wall deficient phenotype as the *mre*-mutants.

CBP025

The *Coxiella burnetii* type IV secretion system effector protein AnkG has to migrate into the nucleus in order to inhibit host cell apoptosis.

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Coxiella burnetii is a Gram-negative, obligate intracellular pathogen that causes Q-fever, a worldwide zoonotic disease. It has been shown that *C. burnetii* employs a type IV secretion system (T4SS), allowing the translocation of effector proteins into the host cell to modulate cellular pathways. Over 60 *C. burnetii* T4SS effector proteins have been identified, mostly with unknown functions. AnkG was the first effector protein with defined function. Thus, it was shown that AnkG inhibits pathogen-induced apoptosis possibly by binding to the pro-apoptotic host cell protein p32 (gC1qR). It was suggested that p32 is involved in mitochondrion-nucleus interaction. Studies with different AnkG-truncations showed that the N-terminus ($AnkG_{1-69}$) is necessary and sufficient for binding to p32 and for apoptosis inhibition.
Here, we demonstrate that ectopically expressed AnkG associates with mitochondria. However, after apoptosis induction AnkG migrated into the nucleus. Intracellular localization and trafficking of AnkG was shown to be dependent on binding to p32, as an AnkG mutant that is unable to bind to p32 co-localized with tubulin and did not traffic to the nucleus after apoptosis induction. Furthermore, $AnkG_{1-69}$, which was shown to be anti-apoptotic, localized to the nucleus, while $AnkG_{70-338}$ which lacks the anti-apoptotic N-terminus, did not localize to the nucleus under all conditions tested. These data suggest that nuclear localization of AnkG might be required for apoptosis inhibition. To determine whether AnkG has to be in the nucleus in order to inhibit apoptosis we generated an AnkG mutant containing a nuclear export signal (NES-AnkG). This mutant displayed a cytosolic localization, even under apoptotic conditions. Furthermore, NES-AnkG was unable to inhibit apoptosis, demonstrating that the anti-apoptotic activity of AnkG depends on nuclear localization. This is in line with our observation that AnkG expression led to an activation of NF-kB, probably by prolonging the duration of p65 in the nucleus.
Taken together, we could show that the intracellular trafficking of AnkG dependents on p32 binding and that the nuclear localization of AnkG is required for apoptosis inhibition. Furthermore, we demonstrated that AnkG expression leads to increased NF-kB activation.

CBP026

Use of nonelectrolytes reveals the channel size and oligomeric structure of the Borrelia burgdorferi P66 porin.

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The outer membrane protein P66 of the Lyme disease spirochete Borrelia burgdorferi forms pores in lipid bilayers with the extremely high single-channel conductance of about 11 nS in 1 M KCl. We studied the diameter of the P66 channel by measuring its single-channel conductance in the presence of different nonelectrolytes with known hydrodynamic radii. Furthermore, we calculated the filling of the channel with these nonelectrolytes. The results suggested that nonelectrolytes with hydrodynamic radii equal or below 0.34 nm pass through the pore, whereas neutral molecules with larger radii only partially filled the channel or were not able to enter it. The diameter of the P66 channel was determined to be 0.8 nm with a constriction site with a diameter of about 0.8 nm. Moreover, the P66 induced membrane conductance could be blocked by about 80-90% by addition of the nonelectrolytes PEG 400, PEG 600 and maltohexaose to the aqueous phase in the low millimolar range. Interestingly, the analysis of the current noise through P66 after its block with these nonelectrolytes demonstrated that no association-dissociation reaction was involved in channel closure. Block of one P66 single-channel conductance unit of 11 nS with PEG 400, PEG 600 or maltohexaose occurred in about eight subconductance states, thus indicating that the P66 channel could be an oligomer of around eight individual channels. The possible organization of P66 as an oligomer was confirmed by Blue Native PAGE and immunoblot analyses, which both demonstrated that P66 forms a complex with a mass of approximately 460 kDa. Second dimension SDS PAGE indicated that P66 was the only component of this pore-forming protein complex.

CBP027

Characterisation of DynA, a BDLP from *Bacillus subtilis*

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Membrane fusion and fission are rapid, dynamic processes that occur in eukaryotic and prokaryotic cells to facilitate transport of vesicles and cargo-trafficking. Members of the dynamin superfamily play an important role in the maintenance of membrane dynamics. These are large GTPase molecules which participate in membrane remodelling events such as vesicle scission, division of organelles, cytokinesis and microbial resistance. According to the Pfam database, around 912 species and 1460 sequences of dynamin-like proteins (DLPs) have been identified in bacteria. DynA is one such DLP found in *Bacillus subtilis*. Its structure is remarkable, as it seems to have developed from a fusion event between two molecules thus consisting of two separate GTPase domains and dynamin-like subunits giving rise to a single polypeptide of 136KDa. Both subunits share a strong intra-molecular co-operativity to facilitate GTPase activity. On account of sequence homology to eukaryotic DLPs and similar biochemical properties, DynA is classified as a member of the dynamin superfamily. It is a bacterial dynamin-like protein (BDLP) that shows nucleotide-independent vesicle tethering and fusion in vitro. DynA seems like a promising BDLP candidate due to the well characterised molecular biology of its host organism and the unique structural features of the molecule.

Biochemical and cell biological characterisation of DynA may provide mechanistic implications for other DLPs, at a single cell level.

CBP028

The magnetosome proteins MamX and MamZ are involved in redox control and are necessary for proper magnetite biomineralization in *Magnetospirillum gryphiswaldense*

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Magnetotactic bacteria use intracellular chains of membrane-enveloped magnetite crystals, called magnetosomes, to orientate along magnetic fields. The sequential steps of magnetosome synthesis involve intracellular differentiation and include membrane vesicle formation, magnetite nucleation and mineralization, as well as magnetosome chain alignment, and are subject to tight genetic regulation. Most of the genes implicated in magnetosome formation are organized in four operons that are clustered within a genomic magnetosome island.
Despite of recent progress in characterization of these genes, the function of the *mamXY* operon has not been well investigated so far. Within this operon, the *mamZ* gene encodes for a unique membrane-spanning protein affiliated to the group of MFS transporters but fused to a putative ferric reductase-like domain, whereas *mamX* may code for a protein belonging to a novel class of cytochromes involved in magnetosome formation.
To get an insight into the function of these proteins, we created unmarked deletions of *mamX* and *mamZ* in the genome of *Magnetospirillum gryphiswaldense*. Both deletion mutants exhibited a similar severe magnetosome biomineralization defect. The mutants still formed regular magnetite particles, however, they also produced poorly crystalline hematite particles, sandwiching the regular crystals and in addition displayed a delay in production of ferrimagnetic magnetosomes. As indicated by fluorescence microscopy and cryo-electron-tomography, magnetosome membrane formation was not affected in the mutants. However, by introducing mutations in putative redox-acting protein domains, we showed that defects are likely caused by disturbance of the redox conditions in the mutants' magnetosome vesicles. Likewise, by omission of nitrate from the growth medium, we further aggravated the phenotype, leading to a predominance of poorly crystalline particles and almost to the absence of regular crystals.
In conclusion, our data suggests that the MamX and MamZ proteins play a major role in magnetite biomineralization. They presumably participate in the same cellular process and are most likely involved in controlling the redox conditions in the magnetosome compartments.

CBP029

Revealing new characteristics of the Tat system component TatA in *Escherichia coli*

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The twin-arginine translocation (Tat) system can transport fully folded proteins. It consists of two membrane-integral protein complex, a TatBC and a TatA complex that transiently interact during translocation. N-terminal signal peptides of translocated protein substrates bear a highly conserved twin-arginine motif that is recognized by the TatBC complex. Thereafter, TatA complexes are recruited to initiate the membrane passage. During early exponential growth, we detected a significant population of TatA in the cytoplasm, forming large complexes as determined by blue-native-PAGE and electron microscopy. Using distinct biochemical in vitro-and in vivo-approaches, we were able to elucidate distinct Tat-specific functions of these soluble TatA complexes that clearly indicate that soluble TatA is not an intermediate on the way to the membrane. We thus could show that TatA has new roles that add to the known role late in the translocation process. Together, the data suggest that Escherichia coli does not differ from other known Tat systems with respect to the presence of most likely functionally important soluble TatA. A general mechanistic model for Tat transport that includes a soluble TatA population will be presented.

CBP030

Conformational transitions during Tat-dependent protein translocation

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Folded proteins can be translocated across prokaryotic cytoplasmic membranes by the twin-arginine translocation (Tat) system. Three membrane-integral components are known to constitute the Tat translocon in *Escherichia coli*, TatA, TatB, and TatC. While TatB and TatC associate to TatBC complexes that have been shown to recognize specific Tat-route-directing signal peptide motifs, the third component forms a complex on its own, the TatA complex. The TatA complex transiently interacts with TatBC/Tat substrate ternary complexes to initiate translocation.
It is an accepted model that TatA somehow facilitates the membrane passage. However, the exact mode of TatA action is enigmatic. In our molecular studies on functions and interactions of TatA, we obtained unexpected evidence for a significant topological change that apparently occurs during translocation. We integrated these data in a mechanistic model for TatA function in which TatA indeed facilitates membrane passage at a late stage of transport.

CBP031

The actin-like MamK cytoskeletal filament drives magnetosome-chain localization and segregation in *Magnetospirillum gryphiswaldense*

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The magnetotactic α-proteobacterium *Magnetospirillum gryphiswaldense* is capable to synthesize magnetosomes, which are unique organelles composed of a core of magnetite (Fe_3O_4) enveloped by a phospholipid bilayer. The magnetosome membrane contains at least 20 proteins involved in magnetosome formation, and they are encoded in a conserved genomic magnetosome island (MAI). In order to navigate along the Earth's magnetic field the magnetosomes are assembled and aligned into a chain. This magnetosome chain has to be divided and equipartitioned to pass on the selective advantage of magnetotaxis to both daughter cells. To ensure proper distribution and equal inheritance of functional magnetosome chains during cytokinesis, the magnetosome chain has to be positioned at the cellular division site, where it is split and separated against the cohesive forces caused by magnetostatic interactions between nascent daughter chains. MamK, an actin-like MAI-encoded protein, polymerizes into a magnetosome cytoskeletal filament holding the magnetosome-chain formation and assembly. Deletion of *mamK* resulted in cells with short and segmented magnetosome-chains and with impaired chain segregation [1] suggesting that MamK mediates magnetosome-chain recruitment to the cell division plane [2]. In order to shed light on how the magnetosome-chain is segregated into the daughter cells and on the role of the MamK cytoskeletal filament in this process, a mutant in mamK was generated. The mutation comprises an amino acid exchange expected to affect the ATPase activity of MamK, thus stabilizing the filament. The mamK mutant displays a WT-like growth and magnetosome biomineralization, forming WT-like magnetosome-chains, and a MamK cytoskeletal filament. However, the mutant displays accentuated impairment of magnetosome-chain segregation. These results suggest that MamK filament dynamics is essential for magnetosome-chain reposition and segregation in *M. gryphiswaldense*.

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CBP032

Functionally important structural characteristics of the TatA membrane anchor

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In *Escherichia coli*, the Tat (Twin-arginine translocation) system is characterized by its ability to transport fully folded precursor proteins with Tat-specific N-terminal signal peptides across the cytoplasmic membrane.

This system consists of the three membrane proteins TatA (or its paralog TatE), TatB and TatC. TatA is a single-spanning membrane protein with its N-terminus in the periplasm and the C-terminus in the cytoplasm. The transmembrane domain (TMD) is located at the extreme N-terminus, followed by a hinge region, an amphipathic helix, and a hydrophilic cytoplasmic domain. In *in silico* analyses, we found conserved sequence characteristics of the TatA membrane anchor region that so far have not been addressed experimentally (1). Consequently, we generated TatA variants with altered physical properties and analyzed structural and functional consequences of these alterations. The experimental data demonstrate new invariable key characteristics of TatA that are of critical importance for its function. In addition, other characteristics that have been assumed to play important roles turned out to be irrelevant. The data provide evidence for distinct functions of the TatA domains and contribute to a mechanistic model that will be presented.

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FTV001

The Molybdenum Storage Protein - a special kind of metalloprotein

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The diazotrophic aerobic soil bacterium *Azotobacter vinelandii* fixes molecular nitrogen via nitrogenases that mostly operate with an iron-molybdenum cofactor. Sufficient amounts of molybdenum (Mo) are ensured by a high-affinity uptake system, which includes a special intracellular protein: the molybdenum storage protein (Mosto)[1]. Mosto can gather up to 120 Mo atoms per molecule and is thereby largely responsible for a substantial growth advantage of Azotobacter, in particular, under Mo deficient conditions [2]. X-ray analysis revealed the existence of various discrete polyoxomolybdate clusters, which are located in pockets and along the threefold axis inside the locked cavity of the hexameric protein. Polypeptide-polyoxomolybdate interactions are characterized by multiple hydrogen bonds and a few covalent bonds [3]. While degradation of the polyoxomolybdate cluster is a time, temperature and pH-driven process [1], formation and stabilization of the polyoxomolybdate clusters requires ATP hydrolysis [4]. Interestingly, under *in vitro* conditions, both processes can run in parallel. Recent results with respect to the role of ATP in polyoxymolybdate formation are presented.

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FTV002

Metallophores as predation factors in *cupriavidus necator*

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Cupriavidus necator is a non-obligate microbial predator that can cause lysis of various Gram-negative and Gram-positive bacteria as well as fungi (1). During growth, this organism secretes a peptidic molecule to capture copper from the environment. This metallophore was shown to be highly toxic against other bacteria and it was proposed to promote the predatory activity of *C. necator* (2).
The aim of this study is to track the copper ligand from *C. necator* and to verify its role in predation. For this purpose, the genome of the predatory *C. necator* strain N-1 was initially screened for genes that could be involved in the biosynthesis of a metallophore. After the identification of a promising gene cluster, the expression of selected genes was tested by reverse transcriptase PCR under different fermentation conditions. Once the appropriate conditions for the production of the putative metallophore had been determined, we developed an isolation strategy that relied on the chrome azurol S assay (3). Spectroscopic analyses enabled the identification of the retrieved compound as the known siderophore staphyloferrin B (4).

We are actually analyzing whether staphyloferrin B is able to coordinate copper and may thus represent the long sought predation factor of *C. necator* or whether its affinity towards metals is restricted to ferric iron.

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FTV003

Seeing more than red - Unexpected prodigiosin pigments in *Streptomyces coelicolor*

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The soil bacterium and model Streptomycete *Streptomyces coelicolor* A2(3) produces the red pigments undecylprodigiosin and streptorubin B [1]. Prodigiosins are formed by diverse bacteria such as *Actinomycetes*, *Serratia* and *Hahella*. Although these red secondary metabolites were isolated at the beginning of the last century, their pyrrolyl dipyrrolylmethene skeleton, was not determined until 1960 [2]. Up to now, several prodigiosins - varying in the alkyl substitution pattern of the pyrrolyl dipyrrolylmethene core - were identified [3].
Although the biosynthesis and the corresponding gene cluster of undecylprodigiosin and streptorubin B seem to be very well understood [4], we report the formation of a series of prodigiosin-derived compounds in *S. coelicolor*. The novel compounds are only formed when *S. coelicolor* is grown under stress conditions.
Under stress *S. coelicolor* exhibits a microdiverse growth. Among the different stress-induced novel phenotypes a bright red one was isolated. Analysis of its metabolic profile by LC-MS revealed a very strong induction of undecylprodigiosin and streptorubin B, and the pronounced formation of additional secondary metabolites in comparison to the wild type. Separation of the crude cell extract by size exclusion, silica and reversed phase chromatography yielded diverse colorful compounds. Although none of the isolated pigments was red, structure elucidation by high resolution mass spectrometry, tandem mass spectrometry and NMR clearly indicated that all of them are related to undecylprodigiosin and streptorubin B.
Thus one will need to revisit the prodigiosin biosynthesis in order to understand how the new prodigiosin derivatives can be formed. It looks like that stress leads to the induction of structural diversity of the prodigiosin biosynthesis.

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FTV004

Biosynthesis of Chlorophylls: Three-dimensional structure of ADP• AlF_3-stabilized protochlorophyllide oxido-reductase complex

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Photosynthesis utilizes chlorophylls for the conversion of light into chemical energy. During chlorophyll biosynthesis, dark-operative protochlorophyllide oxidoreductase (DPOR) catalyzes the chemically challenging reduction of the fully conjugated ring system of protochlorophyllide *a* (Pchlide) to chlorophyllide *a*, the last common precursor of chlorophyll *a* and bacteriochlorophyll *a* biosynthesis [1, 2]. The multi-subunit metalloenzyme DPOR shares significant amino acid sequence and structural homology to nitrogenase. The ATP-dependent subunit L_2 carries a redox-active [4Fe-4S] cluster that transfers electrons to the corresponding [4Fe-4S] cluster of the heterotetrameric subunit $(NB)_2$ which also comprises the Pchlide binding

pocket [3, 4]. DPOR catalysis involves the transient formation of the octameric $(L_2)_2(NB)_2$ complex which can be "trapped" in the presence of non-hydrolyzable ATP analogs [5]. This allowed for the purification and crystallization of the substrate-bound ternary complex from the marine cyanobacterium *Prochlorococcus marinus* [6]. The crystal structure at 2.6 Å resolution reveals that substantial ATP-dependent conformational rearrangements of L_2 trigger the protein-protein interactions with $(NB)_2$ and facilitate the electron transduction *via* redox-active [4Fe-4S] clusters. The protein structure permits a thorough investigation of the interaction surface of L_2 and $(NB)_2$ in comparison to the related nitrogenase complex. Pchlide is bound in a cavity formed mainly by hydrophobic residues of subunits N and B. Mutagenesis experiments identified amino acid residues potentially involved in substrate protonation. The results of these functional studies in combination with the structural analysis of the substrate binding pocket allowed for the deduction of a mechanism for the stereospecific substrate protonation. Finally, we present the identification of artificial "small-molecule substrates" of DPOR in correlation to those of nitrogenase.

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FTV005

Deletion of *rodA* affects peptidoglycan composition in *Staphylococcus carnosus* TM300

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The bacterial cell is surrounded by a cell wall - the peptidoglycan sacculus - which provides strength and shape to the cell. This peptidoglycan is a macromolecule consisting of glycan strands that are cross-linked by peptide side chains. In *Staphylococcus aureus* and *Staphylococcus carnosus*, a pathogenic and an apathogenic representative respectively, cross-linking is indirect via a pentaglycine interpeptide bridge.
The knowledge about the biosynthesis of the peptidoglycan sacculus in Gram-positive cocci and the interplay of the proteins involved is still very limited. The precursor lipid II is synthesized within the cytoplasm and then transported outside the cell where synthesis of the actual peptidoglycan sacculus is catalyzed by the penicillin binding proteins (PBPs). In *E. coli* transport of lipid II across the cytoplasmic membrane was recently shown to be mediated by FtsW, a protein of the SEDS-family (shape, elongation, division, sporulation). In *Staphylococcus carnosus* there are three genes annotated which encode proteins of the SEDS family, amongst them a homologue for RodA. RodA is required for lateral growth of rod-shaped bacteria like *Bacillus subtilis* or *E. coli,* but its role in a coccoid organism like Staphylococcus is not known so far.
We successfully deleted the *rodA* gene of *S. carnosus*. This resulted in a slow growing but viable mutant which is more prone to lysis than the wild type strain. We investigated the sites of active peptidoglycan biosynthesis by microscopy using fluorescent vancomycin which binds to non-cross-linked stem peptides still containing D-Ala-D-Ala. We observed only low binding of vancomycin compared to the wild type labeling. Further investigations of the peptidoglycan of the *rodA* mutant revealed differences in the muropeptide pattern hinting to an incorporation of serine and alanine into the interpeptide bridge. In addition, the mutant strain seems to possess muropeptides which contain a non-amidated glutamate in their stem peptide. Bacterial-Two-Hybrid experiments with the respective *S. aureus* proteins resulted in an interaction of RodA with all four native PBPs.
Taken together these results suggest a guardian role of RodA during peptidoglycan biosynthesis, ensuring the incorporation of amidated lipid II with a pentaglycine interpeptide bridge.

FTV006

Architecture of Smc–ScpAB

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Reliable segregation of replicated chromosomes is a prerequisite for maintaining genomic integrity. SMC-kleisin complexes in bacteria and archaea are important for proper chromosome condensation and segregation and are thus designated "prokaryotic condensins". In eukaryotes, SMC–kleisin complexes called "cohesin" and "condensin" operate during and after DNA replication to promote chromosome segregation in mitosis and meiosis. Prokaryotic complexes are usually of the Smc–ScpAB type and are formed by canonical SMC and kleisin proteins, Smc and ScpA, and the third subunit ScpB. Some branches of γ-proteobacteria including E. coli harbor condensin (MukBEF) formed by deviant homologues MukB, MukE and MukF. Condensin mutations are lethal both in B. subtilis and E. coli under standard growth conditions.
We determined the architecture of Smc-ScpAB type condensins by X-ray crystallography and biochemistry in vitro as well as by site-specific cross-linking and genetics in B. subtilis. Strikingly, we found that homodimeric Smc is asymmetrically bridged by a single ScpA to form huge tripartite rings. The architecture of eukaryotic SMC-kleisin complexes is likely similar to Smc-ScpAB, but highly diverged from MukBEF assemblies. We define a molecular mechanism that ensures asymmetric assembly and conclude that the basic architecture of SMC–kleisin rings has evolved prior to the emergence of eukaryotes.

FTV007

Carboxylation is a common biochemical strategy to activate naphthalene in several anaerobic bacteria.

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Non-substituted aromatic hydrocarbons like naphthalene or phenanthrene seem to be activated by direct carboxylation. Recent studies using the sulfate-reducing enrichment N47 showed the conversion of naphthalene and ^{13}C-labelled bicarbonate to [^{13}C]-2-naphthoic acid providing evidence that anaerobic naphthalene degradation is initiated via direct carboxylation (Mouttaki, 2012). Here, we aim to elucidate if carboxylation is a common activation reaction among different polycyclic aromatic hydrocarbon (PAH) degraders. The tested organisms are NaphS2 (Galushko, 1999), a marine sulphate-reducing delta proteobacterium and N49 (Kleemann, 2011), an iron-reducing enrichment consisting mainly of a Gram-positive bacterium belonging to the family *Peptococcaceae*. N49 was enriched from a contaminated aquifer near Stuttgart. Cell suspension assays for naphthalene carboxylase activity were carried out under strictly anoxic conditions in in a ^{13}C-bicarbonate buffer, pH 7.3 buffer as described previously (Mouttaki, 2012). All assays were performed with whole cells. Naphthalene was added as a saturated solution and the reaction was followed by measuring ^{13}C-2-naphthoic acid formation by LC/MS/MS. The increase in ^{13}C-2-naphthoic acid formation showed that both, the Gram-positive and the Gram-negative strictly anaerobic microorganisms activated naphthalene via a carboxylase reaction similar to the sulfate reducer N47. This new carboxylation reaction of non-substituted aromatic hydrocarbons seems to be a common strategy within different organisms.

Galushko A, Minz D, Schink B & Widdel F (1999) Anaerobic degradation of naphthalene by a pure culture of a novel type of marine sulphate-reducing bacterium. *Environ Microbiol* **1**: 415-420.
Mouttaki H, Johannes J, Meckenstock RU (2012) Identification of naphthalene carboxylase as a prototype for the anaerobic activation of non-substituted aromatic hydrocarbons. *Environ. Microbiol*.,**14**: 2770-2774.
Kleemann R, Meckenstock RU (2011) Anaerobic naphthalene degradation by Gram-positive, iron-reducing bacteria. *FEMS Microbiol Ecol*. **78(3)**:488-96.

FTV008

Impact of the compatible solute hydroxylectoine on cell-free protein synthesis

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Cell-free protein synthesis is of great interest in both applied and basic research [Jewett & Swartz, 2004]. The production of a protein of interest without the limitations of cell growth and the potentially negative influences on the expression system make this technique attractive for high throughput screening and individual applications such as personalized medicine. Furthermore cell-free protein synthesis is the only way to enable the expression of toxic proteins or other so far inaccessible proteins. Because of the increasing importance of this technique measures to improve transcription/translation rates and/or stress stability of the system are in high demand.
Compatible solutes are natural low-molecular mass osmoprotectants of various halophilic bacteria under osmotic stress. In addition to their osmoprotective function they have been shown to stabilize proteins and maintain their biological function. Of the compatible solutes presently under investigation hydroxyectoine attracts particular interest because of its outstanding performance as both dual-stress stabilizer (low water activity and high temperature) and vitrificant (glass-forming solute).
We applied hydroxyectoine in an *E. coli* lysate-based cell-free protein synthesis system with luciferase as the reporter protein according to Seidelt et al. [2009]. We were able to demonstrate that increasing concentrations of hydroxyectoine had a remarkable impact on the performance of the system leading to a two-fold increase at 500 mM. The molecular basis of the interaction of hydroxyectoine with the various components of the *in vitro* protein expression system is currently under investigation.

Jewett MC & Swartz JR (2004) Biotechnol Prog 20: 102-109
Seidelt B et al. (2009) Science: 326:1412-1415

FTV009

ARB phylogenetic sequence analysis suite: a preview on version 6

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Sequencing the ribosomal RNA (rRNA) gene is currently the method of choice for phylogenetic reconstruction, nucleic acid based detection, identification, and quantification of microbial diversity, either as the full cycle rRNA approach, or as descriptive data for pure culture studies. This dependence of both classical microbiology and microbial ecology on the manipulation of rRNA gene sequence data led to the need for capable software environments. The ARB software project was started in response to this need, and is being developed for more than 15 years now at the Technical University of Munich.
The ARB software package was designed as an *in silico* workbench for primarily rRNA gene sequence analysis, but is also extensible to other sequence data. It features an efficient in-memory database to store and manage gigabases of processed primary structure data, sequence associated data such as secondary structure and structured per sequence meta-data. Extensive import and export facilities are complemented by advanced database functions. A set of advanced visualization and editing tools for sequence alignments, secondary structure and phylogenetic trees is also available. ARB offers a comprehensive set of integrated algorithms, including sequence alignment, anomaly prediction, probe design and evaluation, and incremental tree reconstruction. Many of these solutions are unique to ARB, such as the unique maximum parsimony approach. To complete its role as a workbench, ARB also includes an extension system allowing the simple graphical integration of external tools such as e.g. RAxML. Finally, for rRNA analysis, ready-made, comprehensive ARB databases are provided by the SILVA.
This talk will preview the features of the upcoming ARB version 6. In this version, major improvements were made to the graphical user interface and the extension system. Under the hood, memory efficiency has been further enhanced. Among the many new features that were added, tree based sequence clustering, and import of metadata from comma or tab-delimited files can be listed.

FTV010

Interaction networks to analyze gene lists from omics data

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Nowadays omics experiments are commonly used in wet lab practice to identify leads involved in interesting phenotypes. These omics experiments often result in unstructured gene lists of which interpretation in terms of pathways or mode of action is challenging. To aid in the interpretation of these gene lists, we developed PheNetic a decision theoretic method that exploits publicly available information, captured in a comprehensive interaction network to obtain a mechanistic view on the listed genes i. PheNetic selects from the comprehensive interaction network the sub-networks highlighted by these gene lists. We applied PheNetic on a E. coli interaction network to reanalyze a publicly available dataset assessing gene expression of 27 Escherichia coli knock-out mutants under mild acidic conditions. Being able to unveil in an unsupervised way previously described mechanisms involved in acid resistance demonstrated both the performance of our method and the added value of our integrated E. coli network.

FTV011

MAPK signaling and mycoparasitism: transcriptomic and proteomic approaches to dissect the mycoparasitic interaction of the biocontrol fungus *Trichoderma* with phytopathogenic host fungi

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The biological control agent *Trichoderma,* a ubiquitous filamentous fungus, is applied against fungal plant diseases as alternative to synthetic fungicides. As biocontrol by *Trichoderma* includes a direct antagonism of plant pathogenic host fungi by mycoparasitism, the molecular processes underlying this specific fungus-fungus interaction have been studied. Investigations on the underlying intracellular signal transduction pathways of *Trichoderma atroviride* revealed the involvement of a host-dependent MAP kinase in triggering of the mycoparasitic response. Mutants missing the Tmk1 MAPK show infection structures comparable to the parental strain, they over-produce endochitinase42, a key enzyme of mycoparasitism, and show elevated antifungal activity caused by over-production of low molecular-weight metabolites. Despite these enhancements in two of the three mycoparasitism-relevant processes known so far, the D*tmk1* mutant exhibits reduced mycoparasitic activity against host fungi. These findings suggests that additional still unknown genes/proteins and processes are contributing to *T. atroviride* mycoparasitism which were aimed to be identified by using the D*tmk1* mutant as tool. To this end, comparative transcriptomics and proteomics approaches were applied to identify target genes and proteins being regulated by the Tmk1 MAPK upon host recognition and playing key roles in mycoparasitism.

FTV012

Soil eukaryotic unicellular micro-organisms facing crop fertilization: a metagenomic approach

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Soil organic and inorganic fertilizations are commonly used since more than one century to increase yield of cropped fields. Although their effect on plant growth through increase in nutrient availability is obvious, little is known about their impact on belowground microorganisms, particularly on a long term scale. Unicellular eukaryotes, which carry as diverse functions as primary production (microalgae), predation (protists) or decomposition (yeast), are key components in the soil food web and nutrient cycles. As fertilization induce changes in natural nutrient cycles, important modifications in soil communities have to be expected in intensely fertilized soils. Soil DNA from different farmyard manure and mineral fertilization treatments (long term static fertilization experiment, Bad Lauchstädt, Germany) was extracted and served as template to amplified specific markers for fungi (internal transcribed spacer), protists (kinetoplastida, chrysophyceae and cercozoa ribosomal 18S), photoautotrophs (plastid 23S), as well as a general marker for eukaryotes (18S). Subsequent high-throughput pyrosequencing rendered a total of 350 000 good quality sequences which were further clustered in operational taxonomic units (OTUs) and assigned to reference databases (UNITE, SILVA, EMBL). First results indicated that manure fertilization induced significant changes in community composition of all unicellular eukaryotes groups, when mineral fertilization only played a minor role. Co-occurrence patterns of successfully identified abundant OTUs will be further analyzed to identified important link in the microbial food web.

FTV013

Microbes under stress - Slowing down of population recovery as a warning for an approaching collapse.

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For populations under environmental stress, small changes in external conditions may lead to a sudden collapse. Recovery from such a collapse is often difficult: the stressor often has to be reduced to far below the collapse threshold before the system returns to its initial state. Therefore, there is an increasing interest in methods to probe - and optimally, restore - the resilience of a system before it collapses. Theory proposes that there are generic mathematical 'early warning signals' which indicate upcoming transitions in a range of complex systems. For example, recovery rates from small perturbations are expected to warn for an upcoming collapse as they should decrease when a tipping point is approached. However, it is unclear if this early warning signal can be used to predict transitions in real systems.
We used a perturbation experiment to probe the resilience of a light-stressed cyanobacterial population. In this experiment, light-intensity was gradually increased until photo-inhibition drove the population to a collapse when a critical light level was exceeded. Every 4-5 days the population was perturbed by removing 4% of its biomass. Initially, the cyanobacteria recovered rapidly, but in accordance with theory, as the system came closer to the critical point recovery rates became increasingly slow. In addition, autocorrelation of biomass fluctuations rose when the tipping point was approached, which supports the idea that autocorrelation can be used as an indirect indicator of slowing down. Although stochasticity prohibits prediction of the timing of a systems collapse, our results suggest that monitoring recovery rates of experimental or natural perturbations may help to rank systems from resilient to fragile.

FTV014

Absence of cytochrome aa_3 oxidase causes oxidative stress in *Corynebac-terium glutamicum*

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The cytochrome bc_1 complex (*qcrCAB*) of *Corynebacterium glutamicum* was previously shown to form a supercomplex with cytochrome aa_3 oxidase (*ctaD, ctaC, ctaF, ctaE*) [1]. A unique feature of the supercomplex is a diheme cytochrome c_1 (QcrC), the second heme of which takes over the function of a separate cytochrome *c*, which is absent in this species. A Δ*ctaD* mutant lacking the gene for subunit I (CtaD) of the aa_3 oxidase was shown to also lack QcrC, indicating that this protein is unstable in the absence of CtaD. Cytochrome *b* (QrcB) and the Rieske iron-sulfur protein (QcrA) were still detectable in membranes of the Δ*ctaD* mutant [1]. A Δ*qcr* mutant lacking the entire bc_1 complex did not affect the composition and integration of the aa_3 oxidase into the membrane. Astonishingly, the Δ*ctaD* mutant, but not the Δ*qcr* mutant, grew extremely bad in glucose minimal medium, which could hardly be explained by differences in respiratory energy conservation. Further analyses of the Δ*ctaD* mutant revealed a strongly disturbed pattern of fatty and mycolic acids as well as significantly increased amounts of thiobarbituric acid-reactive substances. These results suggest that the residual subunits of the bc_1 complex, i.e. cytochrome *b* and the Rieske iron sulfur protein, could promote the formation of hydroxyl radicals and as consequence lipid peroxidation. Substances with known or proposed antioxidative properties were identified that could reverse the growth defect of the Δ*ctaD* mutant.

1. Niebisch, A. and M. Bott, *Purification of a cytochrome bc_1-aa_3 supercomplex with quinol oxidase activity from Corynebacterium glutamicum - Identification of a fourth subunit of cytochrome aa_3 oxidase and mutational analysis of diheme cytochrome c_1*. J. Biol. Chem., 2003. **278**(6): p. 4339-4346.

FTV015

Symbiont colonization occurs throughout the entire life cycle of hydrothermal vent mussels

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The deep-sea mussel *Bathymodiolus* from hydrothermal vents on the Mid-Atlantic Ridge harbors two types of chemosynthetic bacteria in its gills that provide it with nutrition by gaining energy from oxidizing reduced sulfur compounds and hydrogen (thiotrophic symbionts) as well as methane (methanotrophic symbionts). These intracellular symbionts are housed in specialized gill cells called bacteriocytes. As in all bivalves, *Bathymodiolus* gill tissues grow throughout the mussel's life time raising the question how the new developed gill tissues are colonized by symbionts. This question is particularly interesting because it is assumed that colonization is limited to the early developmental stages of the host in most symbioses between marine invertebrates and intracellular bacteria.
To investigate symbiont colonization of newly formed gill tissues, we used fluorescence in situ hybridization with symbiont-specific probes to analyze semi-thin sections of whole juveniles (5-26 mm shell length) as well as the posterior ends of adult gills where new gill filaments are constantly formed in a so-called budding zone that consists of meristem-like cells. In very small juvenile mussels, we observed that all tissues including the gills, mantel, foot, and adductor muscle contained both types of symbiotic bacteria, while in all juveniles bigger than 5 mm shell length the two symbionts were only found in the gill tissues. This shows that colonization patterns are similar for both symbionts and indiscriminate during early juvenile stages. The specific colonization of gill tissues only occurs at a later developmental stage. Our analyses of the posterior end of both juvenile and adult gill tissues revealed that all gill filaments except the first freshly formed 7 to 9 filaments as well as the budding zone harbored symbionts. Newly formed gill tissues are thus originally symbiont free and only later become infected with the two symbionts as they extend and differentiate. A life long de novo colonization by endosymbionts of aposymbiotic host cells has, to our knowledge, not been previously shown in animals with intracellular bacteria. These colonization patterns make *Bathymodiolus* an ideal model for examining the patterns and processes of symbiont uptake and tissue specificity.

FTV016

Freshwater actinobacteria: From uncultivated to enigmatic

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Actinobacteria within the acI lineage are often numerically dominating freshwater ecosystems, where they can account for >50% of total bacteria in the surface water. Despite their importance there are no reports on the isolation of acI. We thus set out to use single cell genomics to gain insights

into their genetic make-up, with the aim of learning about their physiology and ecological niche. From the single cell genome, a representative from acI-B1 was analysed and the metabolic reconstruction gave us some hints about the niche of this organism. With this information, enrichments were established and after a few months we were able to obtain a highly enriched co-culture of acI-B2. To our surprise acI-B2 grows in high abundance and quite fast together with other highly abundant freshwater bacteria,*Polynucleobacter sp*. However to detect the growth of both organisms a qPCR assay was necessary, since the density of the culture is relatively low. Some tests have been done on these co-culture and some interesting features of acI have been confirmed. There are still many questions that remain to be answered for acI and one of the most intriguing would be the character of its interaction with*Polynucleobacter sp*. or other bacteria in the environment.

FTP002

Trehalose-phosphate synthases of *Rhodococcus*

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The actinobacterium *Rhodococcus opacus* 1CP has been described for its ability to produce a novel type of trehalose dinocardiomycolate. The formation of these surface-active trehalose lipids strongly depends on the utilization of long-chained *n*-alkanes as sole substrates. Trehalose-6-phosphate was described to be a central intermediate in biosynthesis of those glycolipids. As a first entry to investigate the formation of the trehalose dinocardiomycolate in strain 1CP, the biosynthetic routes leading to trehalose-6-phosphate were investigated.
Two gene variants, *otsA1* and *otsA2*, encoding different putative trehalose-6-phosphate synthases were identified in strain 1CP. By recombinant gene expression the respective proteins were obtained and characterized for their catalytic properties and (co)substrate spectra. Both OtsA-variants showed a similar preference for the glucosyl acceptor glucose-6-phosphate. But, distinct differences were obtained for the glucosyl nucleotide donors, especially for ADP- and UDP-glucose. In addition, a different extent of activation was observed for OtsA-variants in the presence of heparin.
Obtained data were compared to wild-type OtsA enrichments from glucose as well as from *n*-alkane-grown 1CP-biomass. Evidence was provided that dominantly OtsA2 is expressed during growth on glucose, whereas OtsA1 seems to be specifically involved in the *n*-alkane-induced formation of the trehalose dinocardiomycolates. The physiological role of OtsA2 is therefore likely to be in the formation of the compatible solute trehalose, a function for which an additional enzyme OtsB is recruited for.
In summary, both OtsA1 and OtsA2 show considerable similarity to trehalose-phosphate synthases of other Actinobacteria, especially Mycobacterium species. But this was the first time that two *otsA*-genes from one strain were investigated, found to be active, and are evidently involved in different physiological roles.

FTP003

Bacteriological quality of indoor air environment in private and government owned abattoirs in Benin City, Nigeria

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The bacteriological quality of indoor air environment of two abattoirs, private and government owned abattoirs in Benin City was studied using the settle plate methods. The air samples were collected monthly for a period of six months, between October, 2011 and March, 2012. The mean airborne bacterial counts grown on nutrient agar in the private and government abattoirs ranged from 8.47 ± 316.78 x 10^2 cfu/m^3 to 5.225 ± 574.37 x 10^3 cfu/m^3 during slaughtering and 6.376 ± 106.94 x 10^2 cfu/m^3 to 1.0766 ± 1749.6 x 10^4 cfu/m^3 after slaughtering. The mean airborne bacterial counts grown on blood agar in the private and government abattoirs ranged from 4.540 ± 181.63 x 10^2 cfu/m^3 to 3.115 ± 68.11 x 10^3 cfu/m^3 during slaughtering and 3.750 ± 100.36 x 10^2 cfu/m^3 to 1.091 ± 277.11 x 10^3 cfu/m^3 after slaughtering. The mean airborne bacterial counts grown on eosine methylene blue agar in the private and government abattoirs ranged from 3.370 ± 44.18 x 10^2 cfu/m^3 to 2.108 ± 32.46 x 10^3 cfu/m^3 during slaughtering and 1.060 ± 56.23 x 10^2 cfu/m^3 to 1.318 ± 325.06 x 10^3 cfu/m^3 after slaughtering. The result revealed the isolation of seven airborne bacterial isolates which include *Staphylococcus aureus*, *Staphylococcus epidermidis, Streptococcus* sp., *Bacillus* sp., *Pseudomonas* sp., *Klebsiella* sp., and *Escherichia coli*. The frequency of occurrence and distribution of the airborne bacterial isolates revealed *Staphylococcus. aureus* (48.75%) and *Bacillus* sp. (28.68%) as the most frequently isolated airborne bacterial isolates in the private abattoir, while *S. aureus* (31.95%) and *Bacillus* sp. (41.93%) were also recorded as the most frequently isolated airborne bacterial isolates in the government abattoir. The statistical analysis showed no significant difference between the airborne bacterial counts recorded during and after slaughtering in the two abattoirs. The result of the plasmid determination revealed that the airborne bacterial isolates harboured plasmid sizes which ranged between 1.5 kbs to 25.2 kbs. This work demonstrated that the quality of air in the abattoirs is influenced by the population and type of airborne bacterial isolates, which play significant role in the level of hygiene in meat processing plants. The type and population of airborne bacterial isolates in the indoor air of the two abattoirs are of great concern, which serves as index of quality of air in the environment.

FTP004

The crystal structure of galactitol-1-phosphate 5-dehydrogenase from *Escherichia coli* K12 provides insights into its anomalous behavior on IMAC processes

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Endogenous galactitol-1-phosphate 5-dehydrogenase (GPDH) (EC 1.1.1.251) from *Escherichia coli* spontaneously interacts with Ni^{2+}-NTA matrices becoming a potential contaminant for recombinant, target His-tagged proteins. Purified recombinant, untagged GPDH (rGPDH) converted galactitol into tagatose, and D-tagatose-6-phosphate into galactitol-1-phosphate, in a Zn^{2+}- and NAD(H)-dependent manner and readily crystallized what has permitted to solve its crystal structure. In contrast, N-terminally His-tagged GPDH was marginally stable and readily aggregated. The structure of rGPDH revealed metal-binding sites characteristic from the medium-chain dehydrogenase/ reductase protein superfamily which may explain its ability to interact with immobilized metals. The structure also provides clues on the harmful effects of the N-terminal His-tag.

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FTP005

New insights in underground talking: *Tricholoma vaccinum* and the mycorrhizosphere

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The ectomycorrhiza between the widespread basidiomycete *Tricholoma vaccinum* and its tree host spruce (*Picea abies*) represents a model system for mutualistic interactions and interspecies communication. Other soil fungi can produce morphogenic substances, which modulate the ectomycorrhizal talking and furthermore growth and physiology of the individual partners and the established mycorrhiza. Different mating types of Zygomycetes belonging to the *Mucorales* communicate *via* a system based on apocarotenoids. An early intermediate after the cleavage of β-carotene is a C_{18}-ketone, called D'orenone, which interferes with the root development of *Arabidopsis thaliana* (Schlicht *et al*., 2008). In this work the effects of D'orenone on the spruce root system, the fungal partner *T. vaccinum* and the symbiosis were tested. Moreover, we studied the protein expression during D'orenone treatment with 1D- and 2D-PAGE. D'orenone modulated the spruce root architecture differently than in *A. thaliana*. D'orenone reduced the biomass but, interestingly, increased the measurable concentration of the phytohormone indole-3-acetic acid in *T. vaccinum*. Furthermore, D'orenone changed the cytosolic protein expression in *T. vaccinum*. These results show that fungal metabolites can affect, secondarily,relationships in soil, like mycorrhiza. With the newly sequenced genome of *T. vaccinum* the search for D'orenone receptors started and experiments with the mycorrhized tree under D'orenone treatment. Additionally, we will screen for new mycohormones by co-cultivating *T. vaccinum* with rhizospheric fungi and spruce root exudates. More information about the impact of mycohormones will improve the knowledge about communication in the mycorrhizosphere

affecting tree morphology, mycorrhization rate, and, thereby, influence tree health and whole ecosystem fitness.

Schlicht. M., Samajová, O., Schachtschabel, D., Mancuso, S., Menzel, D. Boland, W., Baluska, F. 2008. *The Plant Journal* 55, 709-717

FTP006

New reactions in anaerobic alkane and alkene metabolism

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Hydrocarbons are microbially degraded under aerobic and anaerobic conditions. Particularly the anaerobic degradation of alkanes and alkenes is a current research topic with many open questions.
Desulfococcus oleovorans and *Desulfatibacillum alkenivorans* are well-known sulfate-reducing bacteria degrading alkenes or alkanes anaerobically. Alkane metabolism of *D. alkenivorans* is initiated by fumarate addition to the subterminal methylene group, leading the formation of even-chain fatty acids from even-chain alkanes (1). In contrast, *D. oleovorans* is of special interest as model strain for a different, yet unknown alkane metabolic pathway leading to odd-chain fatty acids from even-chain alkanes. It was proposed that the initial step of the alkane degradation by *D. oleovorans* is a carboxylation reaction at C3 of the alkane chain, resulting in a hypothetical intermediate that is further degraded to an activated fatty acid one carbon atom shorter than the primary alkane (2).
We are working on identifying and characterizing the enzymes of the unknown anaerobic alkene and alkane metabolic pathways in these sulphate reducing bacteria. We propose that the initial step of the unknown alkane degradation pathways of *D. oleovorans* is a hydroxylation at C2, which may be catalyzed by an enzyme with high sequence identity to ethylbenzene dehydrogenase that is induced in alkane-degrading cells of *D. oleovorans*. Ethylbenzene dehydrogenase catalyzes the initial oxygen-independent hydroxylation of anaerobic ethylbenzene degradation in *Aromatoleum aromaticum* (3). Therefore, we investigate whether the enzyme from *D. oleovorans* catalyzes an analogous hydroxylation reaction with alkanes. We are also currently investigating alkene-grown cells for the presence of induced proteins related to this pathway.

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FTP007

Structure and reaction mechanism of benzylsuccinate synthase and its activating enzyme

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The degradation of toluene under anaerobic conditions is initiated by a stereospecific addition of the methyl group of toluene to the double bond of the co-substrate fumarate, resulting in the formation of (*R*)-benzylsuccinate as the first intermediate of the metabolic pathway. This reaction is catalyzed by a member of the glycyl radical enzyme family, (*R*)-benzylsuccinate synthase (BSS), which is posttranslationally activated by an S-Adenosylmethionine dependent activating enzyme, BssD. The three different subunits of BSS, α, β, and γ, with molecular masses of 98, 8.5 and 6.4 kDa, respectively, compose a hexameric structure with an $\alpha_2\beta_2\gamma_2$ composition, resulting in a molecular mass of 220 kDa. The large α-subunits of BSS contain the essential glycine and cysteine residues that are conserved in all glycyl radical enzymes. The two smaller subunits contain 4Fe4S-clusters with yet unknown function.
The structural genes, *bssC, A* and *B*, coding for the three subunits of BSS (α, β and γ) are arranged in the toluene-inducible *bss*-operon together with the gene coding for the activating enzyme, *bssD*, and at least four other genes *bssE, F, G* and *H* coding for hypothetical proteins of unknown function.
The mechanism of BSS is predicted to involve the formation of an enzyme-bound substrate radical derived from the addition of toluene to fumarate to form a product-related radical. This radical re-abstracts the hydrogen atom from the enzyme, resulting in the final product of the reaction, benzylsuccinate, and a regenerated radical-containing enzyme.
In our project, we want to clarify the roles of the hypothetical proteins BssE - BssH by using genetic approaches. Furthermore we will obtain further information about the mechanism of BSS reaction by biochemical, structural and spectroscopic investigations of the activated enzyme both in wild type cells and with homologously overproduced mutant BSS variants. For example, we identified new organic radical species via EPR spectroscopy by using different substrate analoga and inhibitors and try to relate this information to mechanistic details of the BSS reaction mechanism.

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FTP008

Novel ligands for a DctP-Family substrate binding protein of a TRAP transporter in *Advenella mimigardefordensis* strain DPN7^T

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Substrate binding proteins (SBP) of primary and secondary transporters enable high affinity uptake of solutes in bacteria. The tripartite ATP-independent periplasmic (TRAP) transporters are the best investigated tripartite transport system in secondary solute transport. The first characterized system of the TRAP transport family was the DctPQM system of *Rhodobacter capsulatus*. Since then the transport of several organic acids and few other organic anions by TRAP transport systems has been described [1].
In this study, we focus on a specific SBP ($DctP_{Am}$) of a conspicuous TRAP transport system in *Advenella mimigardefordensis* strain DPN7^T. $DctP_{Am}$ was shown to be essential for the transport of five monosaccharides and one organic acid. Deletion of $dctP_{Am}$ impaired growth of *A. mimigardefordensis* strain DPN7^T if cultivated on mineral salt medium supplemented with D-glucose, D-galactose, L-arabinose, D-fucose, D-xylose or gluconic acid, respectively, as sole carbon source. Up to now, carbohydrate transport by a TRAP transport system has not been described for monosaccharides like glucose; instead, the transport of monosaccharides in most bacteria is performed by PTS systems, ABC transporters or diffusion via major facilitator proteins. The TRAP mediated transport of gluconic acid has been unraveled by Steele *et al*. in 2009 [2]. Here, we show for the first time that the transport of some monosaccharides in *A. mimigardefordensis* strain DPN7^T is undoubtedly performed by a TRAP transport system.

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FTP009

Characterization of the wax ester synthase/acyl-CoA: diacylglycerol acyltransferase from *Acinetobacter baylyi* as a model enzyme for wax ester and triacylglycerol biosynthesis

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Synthesis of triacylglycerols (TAG) and wax esters (WE) is catalyzed by wax ester synthase/acyl-CoA:diacylglycerol acyltransferases (WS/DGAT) in bacteria. Database searches indicate a broad distribution of homologous enzymes among eukaryotic protists, plants and animals. WE, composed of a long-chain acyl and a fatty alcohol residue, are valuable ingredients of many commercial products. However, natural sources for high-quality WE are currently mainly restricted to the expensive oil of the jojoba plant. The Gram-negative *Acinetobacter baylyi* is able to accumulate significant amounts of jojoba-oil like WE and minor amounts of TAG as energy and carbon reserve. The responsible key enzyme AtfA represents the best studied acyltransferase of the WS/DGAT family, so far, and serves as model enzyme for this wide-spread and largely unexplored enzyme family. Unfortunately, its three-dimensional structure and exact biochemistry still remain mostly unknown. Therefore, different approaches to gain further insights into the relationship between sequence, structure and function of AtfA were pursued. As a first step, essential amino acids could be identified by random mutagenesis. Furthermore, multiple sequence alignments as well as *in silico* studies revealed conserved amino acid patterns and secondary structural elements of this enzyme family. On the basis of these first insights, site-directed mutagenesis and structure analysis of the model

enzyme AtfA will not only contribute to understand the general functionality of this type of acyltransferase, but also enable future enzyme optimization approaches to promote a sustainable biotechnological production of WE (and TAG) with desired chemical compositions.

FTP010
OmpW of *Caulobacter crescentus* functions as an outer membrane channel

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Caulobacter crescentus is a gram-negative bacterium found in oligotrophic aquatic environments such as soil and fresh water and is widely used as a model organism for the studies of bacterial cell cycle differentiation. It is an unusual gram-negative bacterium in that genes coding for typical general diffusion outer membrane porins of the OmpF/C type have not been identified in its genome. Similarly, genes coding for specific porins such as Tsx or LamB are also absent. Instead, the genome of *C. crescentus* contains a large number of genes that code for TonB-dependent receptors. Surprisingly, a high channel-forming activity was observed with crude outer membrane extracts of *C. crescentus* in lipid bilayer experiments indicating that its outer membrane contained an ion-permeable channel with a low single-channel conductance of about 125 pS in 1 M KCl. The channel-forming protein has a molecular mass of about 22 kDa. From partial protein sequencing the channel-forming protein was identified to be a member of the OmpW family of outer membrane proteins from gram-negative bacteria. Biophysical analysis of OmpW of *C. crescentus* suggested that it has features that are not typical for gram-negative bacterial porins because it is not wide and water-filled. Homologues of OmpW of *C. crescentus* with known 3D-structures are OmpW of *E. coli* and OprG of *Pseudomonas aeruginosa*, which both have a very low permeability if any. A comparison of the primary, secondary and tertiary structures of the different OmpW homologues suggested that W155 of OmpW of *E. coli* and W170 of OprG could be the plug of the 8 beta-barrel outer membrane proteins of *E. coli* and *P. aeruginosa*. To study this in detail, the gene coding for OmpW from *C. crescentus* was cloned into *E. coli* expression vector pAraJS2 and the protein was expressed in *E. coli* BL21(DE3) omp8ΔompW. Similarly, OmpW mutant protein (K159W, K160Y) from *C. crescentus*, OprG mutant protein (W170K) from *Pseudomonas aeruginosa* and ompW mutant protein (W155K) from *E.coli* are also going to be created and expressed in *E.coli* BL21(DE3) omp8 ΔompW to study their channel-forming properties in lipid bilayer experiments.

FTP011
Formation and alteration of carbonates by microbial activity

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Calcification is a general phenomenon which appears in different environments such as soils and water. Various studies have shown examples for biotic and abiotic mineralization. Abiotic formation of carbonates is influenced by different factors such as temperature and changes in pH. Processes of biomineralization affected by the activity of organisms are known to occur through activity of gastropods or mussles with shells of carbonates, or by bacteria able to modify carbonates by creating an alkaline environment. The processes involved in increasing the pH are associated with the activity of enzymes such as urease and carbonic anhydrase. In this study, the microbial diversity in limestone was investigated, followed by experiments for the detection of biomineralization. The Thuringian Basin, which is located in the central part of Germany, belongs to the Triassic period and is surrounded by layers of Muschelkalk. The investigated sampling site was the quarry Bad Kösen that consists of the Middle Muschelkalk (Karstadt Formation) and the Lower Muschelkalk (Jena Formation). For the isolation of bacteria, rock samples were taken from the Lower Muschelkalk. The cultivation on different media demonstrated low cfu of $3*10^4$ which is typical for rocks as extreme habitats. All in all, 40 strains could be isolated which were cultivated on calcium containing media to study their ability to form or degrade carbonates or to analyze urease activities.

FTP012
Removal of DNA degrading and DNA modifying enzymes from a *Salmonella* vaccine strain in order to improve its suitability for further strain modification

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Live attenuated *Salmonella* vaccines have a proven track record in human and veterinary medicine. Conventionally, vaccine strains have been generated by undirected mutagenesis. Most vaccine strains have been attenuated only in a few genes to render them non-pathogenic. However, like the original isolates these vaccine strains are genetically instable and are proficient to persist in the environment. In order to eliminate such unwanted properties we aim at the removal of bacterial determinants which mediate these phenotypes. Targeted genetic engineering of bacterial genomes has been greatly facilitated by new techniques such as the use of the Red recombinase of bacteriophage Lambda. The efficiency of Red mediated gene replacement in the bacterial chromosome is dependent on transformation of a linear DNA fragment with terminal homology regions matching the chromosomal target locus. The efficiency of this transformation step is hampered by several DNA degrading and DNA modifying enzymes. By multiple deletions of genes encoding such enzymes we have stepwise improved the transformation efficiency of the attenuated *Salmonella enterica* serovar Typhimurium vaccine strain SL7207. Mutant strains were subsequently checked *in vitro* and *in vivo* and found to retain their invasiveness and immunogenicity.

FTP013
Spontaneous grape must fermentation: microbial succession and the influence of must components

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In order to investigate the reasons for sluggish or stuck fermentation, the succession of microorganisms as well as the chemical parameters were studied in parallel during spontaneous fermentation in a german vinery. The sampling took place in regular intervals during a period of several months. The tested musts were from different vineyards and vintages. They were stored in wooden casks as well as in steel casks.
In one wooden cask with stuck fermentation, the main yeast strain during the stuck was *Saccharomyces bayanus*. However, it was replaced by a hybrid strain *Saccharomyces cerevisiae* x *kudriavzevii* when the fermentation continued. This hybrid is supposed to be advantageous for winemaking in the middle of europe due to the combined characteristics of both parents, e.g. tolerance against high ethanol concentration and high osmolarity of *S. cerevisiae* and tolerance against cool temperatures of *S. kudriavzevii* (Gonzàlez et al., 2006).
In addition, a substance with an inhibiting effect on yeasts was found in the young wine of this wooden cask.
This substance shows a high temperature tolerance and does not belong to the known group of killer toxins.

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FTP014
Characterization of the Protopor-phyrinogen IX oxidase from the genus *Leishmania*

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The tetrapyrrole heme is a complex macromolecule and functions as a highly essential prosthetic group for most life on earth. It serves as cofactor in numerous proteins involved in fundamental biological processes such as respiration, photosynthesis and cellular redox reactions, including antioxidant defenses and at several stages of the electron transport chain in prokaryotes and eukaryotes. Heme also serves as a sensor and transport molecule for oxygen. Its complex biosynthesis is catalyzed by a

multienzyme pathway starting from simpler precursors. The penultimate step in heme biosynthesis generates protoporphyrin IX *via* the catalytic conversion of protoporphyrinogen IX by the enzyme protoporphyrinogen IX oxidase (PPO). Most organisms entertain either one of the two known PPOs: in eukaryotes and gram positive bacteria the FAD containing PPO encoded by the gene *hemY*, while in gram negative bacteria the FMN containing PPO encoded by *hemG* is used. However, the eukaryote *Leishmania* sp., devoid of the gene *hemY*, carries a *hemG*-gene on its genome, suggesting *Leishmania* sp. the so far only eukaryote using this route of PPO conversion. Our approach was to verify *Leishmania* sp. PPO acvtivity by complementation of an *E. coli hemG* knock-out mutant. The cellular levels of heme and its precursors, protoporphyrin IX (PROTO) and coproporphyrin III (COPRO), were confirmed by separation on a reversed-phased C-18 column chromatography while recording heme and its precursor metabolites using UV/Vis and fluorescence spectroscopy. To calculate differences in growth behavior between the *E. coli hemG* knock-out mutant and the *E. coli hemG* knock-out mutant complemented with *Leishmania* sp. *hemG* growth kinetics were performed and phenotypical alterations were documented. Further biochemical characterization of the recombinantly overproduced *Leishmania* sp. HemG and crystallization attempts will elucidate the *modus operandi* of this as of yet uncommon PPO.

FTP015

Bacterial metabolism of the organosulfinate hypotaurine

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Hypotaurine (2-aminoethanesulfinate) is a precursor of taurine (2-aminoethanesulfonate) in the taurine-biosynthetic pathway (1) and an osmolyte at high concentrations in deep-sea invertebrates (2;3). Hypotaurine (HT) is known to be utilized by bacteria as a sole source of carbon and energy, however, the corresponding degradation pathway remained unknown (4).
Paracoccus denitrificans PD1222 is able to grow with HT and releases the HT-sulfur as sulfite, and the HT-nitrogen as ammonium, in quantitative amounts, respectively. Complete substrate disappearance and product formation was confirmed using an HPLC-HILIC-ELSD method. Enzyme assays using cell extracts showed that an inducible HT:pyruvate-aminotransferase (Hpa) catalyzes deamination of HT. Partial purification of the Hpa-activity in combination with peptide fingerprinting-mass spectrometry (PF-MS) identified an Hpa-candidate gene as well as an aldehyde-dehydrogenase (ADH) candidate gene. The same two genes were identified *via* 2D-gelelectrophoresis and PF-MS, overexpressed in *E.coli* and purified. The Hpa showed HT:pyruvate-aminotransferase activity, whereat acetaldehyde and sulfite could be identified as the reaction products, but not sulfinoacetaldehyde. The ADH oxidized acetaldehyde to acetate in a NAD^+-dependent reaction.
Based on these results the following degradation pathway for hypotaurine in strain PD1222 can be depicted: In the first step, the identified aminotransferase deaminates hypotaurine to sulfinoacetaldehyde. Sulfinoacetaldehyde is instable and desulfinates spontaneously to acetaldehyde and sulfite. The inducible aldehyde-dehydrogenase oxidizes acetaldehyde to acetate which is the substrate for central metabolism, and sulfite is excreted.

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FTP016

Construction and comparison of *lux*-based bioreporter systems for the detection of alkylquinolone-converting enzymes

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The opportunistic pathogen *Pseudomonas aeruginosa* regulates its virulence via a sophisticated quorum sensing network incorporating *N*-acylhomoserine lactones as well as 2-alkyl-4(1*H*)-quinolones (AQs). 2-Heptyl-3-hydroxy-4(1*H*)-quinolone (the *Pseudomonas* quinolone signal, PQS) and 2-heptyl-4(1*H*)-quinolone (HHQ) were identified as autoinducers in quorum sensing, acting as effectors of the LysR-type transcriptional regulator PqsR [reviewed in 1].
In a preceding study, a *lacZ*-based *P. putida* KT2440 bioreporter strain was constructed, which is highly sensitive towards HHQ (EC_{50} 1.50 ± 0.25 μM) and PQS (EC_{50} 0.15 ± 0.02 µM) [2]. Since β-galactosidase assays are hardly suitable for high-throughput screening, we developed novel *lux*-based bioreporter strains. One approach involves the integration of a cassette, containing a transcriptional fusion of the *pqsA* promoter to the *luxCDABE* operon and the *pqsR* gene constitutively expressed from the *tac* promoter, into the *P. putida* KT2440 genome. The regulator PqsR binds to the *pqsA* promoter in the absence or presence of its effectors PQS or HHQ; however, activation of transcription is greatly enhanced by the PqsR-effector complex [1]. In another approach, *P. putida* KT2440 and *E. coli* DH5α are transformed with plasmids containing the $pqsA_P$::*luxCDABE* fusion and the *pqsR* gene. In the recombinant *E. coli* strain, the transcription of the *pqsR* gene is controlled by a rhamnose-inducible promoter, whereas in *P. putida* KT2440 *pqsR* is constitutively expressed from lac_P. The response and sensitivity of the chromosomal and plasmid-based bioreporters to PQS and HHQ will be compared.
To test the suitability of the bioreporter strains for the detection of AQ-converting enzymes, the *hod* gene from *Arthrobacter* sp. Rue61a encoding the 2-alkyl-3-hydroxy-4(1*H*)-quinolone 2,4 dioxygenase (Hod) is coexpressed. Hod converts PQS to *N*-octanoylanthranilate and carbon monoxide [3]. To identify novel enzymes, genomic libraries will be established in the most suitable bioreporter, e.g., a library of *Arthrobacter* sp. Rue61a, which is able to convert PQS even in the absence of *hod*.

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FTP017

Mutational analysis of a salicylate converting gentisate-1,2-dioxygenase from *Pseudaminobacter salicylatoxidans*

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The bacterium *Pseudaminobacter salicylatoxidans* synthesizes a modified gentisate 1,2-dioxygenase which cleaves several monohydroxylated aromatic compounds, such as salicylate, substituted salicylates, and 1-hydroxy-2-naphthoate[1].The enzyme belongs to the cupin superfamily and the holoenzyme consists of 4 identical subunits which each contain one catalytically active Fe(II)-ion complexed by 3 histidine residues. Recently, the crystal structures of several enzyme-substrate adducts have been determined[2].
We are analyzing the molecular basis for the unique ability of this dioxygenase to oxidatively cleave a wide range of monohydroxylated aromatics. Therefore, several amino acid residues which are presumably involved in the binding of the catalytically active ferrous iron ions, substrate binding or catalysis were changed by site-directed mutagenesis and the enzyme variants analyzed for the conversion of gentisate, 1-hydroxy-2-naphthoate, and (substituted) salicylate(s). Furthermore, the binding of ferrous iron to the enzyme and the effects of several divalent cations on enzyme activity were analyzed.
Several enzyme variants were identified which either completely lost the ability to convert all substrates tested or which demonstrated significant changes in substrate specificity. Thus, several variants were identified, which were unable to convert salicylate but still retained the ability to oxidize gentisate. The combination of these experiments with the crystal structures of the wild-type enzyme and some enzyme variants allowed us to propose some specific traits which might confer to the "salicylate 1,2-dioxygenase" the ability to oxidatively cleave monohydroxylated substrates.

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FTP018

Chemists like it short: Chemoenzymatic synthesis of complestatin

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Secondary metabolites produced by bacteria are a potent source for the development of new drugs. For in-depth studies on molecular targets and clinical trials it is essential to produce such substances in adequate amounts. The non-ribosomal peptide complestatin derived from *Streptomyces lavendulae* is a promising anti HIV lead structure that acts as retroviral integrase inhibitor.[1] Unfortunately its chemical total synthesis is a lengthy and complex process suffering from low yields. Especially the assembly of the biaryl and biaryl ether bonds is critical.[2]
An elegant way to potentially overcome this problem is by combining chemical and enzymatic methods.[3] *In vivo* the two Cytochrome-P450 enzymes ComI and ComJ perform the biaryl coupling reactions leading to complestatin.[4,5] Employing these enzymes *in vitro* along with a synthetic linear complestatin-precursor peptide as substrate would lead to the final molecule with much less effort as the longest linear synthetic sequence would be reduced to only 8 steps. Here we report on the first results of our endeavors to establish such a chemo-enzymatic synthesis of complestatin.

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FTP019

Improving the biogas yield from marine macro algae ("Treibsel") with a heat/acid pretreatment using a waste management concept

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Background: There is a growing interest in biogas energy as a result of increased global demand for energy and shortage of finite energy sources. Commonly used substrates in biogas plants are energy crops which provide generally high biogas yields and show good degradation performance. However, the extensive use of energy crops seems to carry along serious issues e.g. nutrient removal in the soil. Marine macro algae offer a vast substrate source for biogas production and could circumvent many of the facing problems. Macro algae biomass ("Treibsel" or "Teek") accumulating on beaches and shores is considered disturbing, especially in touristic areas. Consequently, algae have to be costly removed in order to maintain a "clean" locality. In most of the cases no sustainable disposing strategy is established and the biomass is dumped inadequately.
Research and Aim: The macro algae conversion to biogas shows greater challenges compared to the degradation of its terrestrial counterparts. A mechanical and physico-chemical pretreatment step can facilitate the molecular fracturing and therefore the conversion to biogas. I carried out experiments using batch fermenter setups testing different heat and acid pretreatment conditions applied on brown algae. Pretreatment previously to methanization is aiming to increase the biogas yield and enhance the degradation of the algae. Laboratory results have shown that an increase in biogas yield mounting up to 60 % is possible, depending on the heat input and acid strength.
Additionally, this work proposes a biogas generation/waste management concept. I tried to implement the recovery of waste heat and industrial acid waste for the pretreatment step to optimize bio-availability and to use "Treibsel" as biomass substrate. Theses and further benefits should create a sustainable whole-process concept providing a one-term solution for various existing problems.
Summary: It seems that "Treibsel" macro algae substrate is suitable for biogas generation and physico-chemical pretreatment can increase the biogas yield significantly. Compared to energy crops, however, the biogas yield is low and regarding this criterion not competitive. Considering further synergetic factors e.g. environmental criteria, waste management and biomass availability a possible sustainable scenario could be developed.

FTP020

The degradation pathway for xenobiotic 4-sulfophenyl-carboxylates in *Comamonas testosteroni* KF-1

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Xenobiotic sulfophenylcarboxylates (SPCs) are formed as intermediates during degradation of laundry surfactant linear-alkylbenzenesulfonates (LAS) [*1*], which are mineralized by heterotrophic bacterial communities in two steps [2]. In a first step, the alkyl side chains of LAS are broken down and SPCs are formed, one of which is 3-(4-sulfophenyl)butyrate (3-C_4-SPC). In a second step, the SPCs are completely degraded, with 3-C_4-SPC being utilized by *Comamonas testosteroni* KF-1 [*3,4*]. For the latter pathway, a first C_2 unit is thought to be cleaved off in form of acetyl-CoA to yield 4-sulfoacetophenone, which is oxygenated to 4-sulfophenyl acetate and subsequently hydrolyzed to acetate and 4-sulfophenol (SP) [*4*]; the corresponding Baeyer-Villiger monooxygenase (BVMO) and carboxyl ester hydrolase have recently been identified and characterized [*5*]. Furthermore, in SP grown cells, a highly inducible 1,2,4-trihydroxybenzene dioxygenase was identified and characterized. Hence, the 3-C_4-SPC degradation pathway in *C. testosteroni* KF-1 is likely to involve a desulfonating SP 3,4-dioxygenase system to yield 1,2,4-trihydroxybenzene from SP, and after an *ortho*-ring cleavage of 1,2,4-trihydroxybenzene maleylacetate is formed, which can then enter central metabolism.
Here we present data of the newly characterized reaction steps of a degradation pathway in *C. testosteroni* KF-1 that represents an example of the adaptation of bacteria for the utilization of xenobiotic compunds as novel growth substrates.

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FTP021

Characterization of the potential heme chaperone HemW

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Modified tetrapyrroles are complex macrocycles and the most abundant pigments found in nature. They play a central role in electron transfer dependent energy generating processes such as photosynthesis and respiration. They further function as prosthetic groups for a variety of enzymes, including catalases, peroxidases, cytochromes of the P450 class and sensor molecules. Heme is a hydrophobic molecule and associates non-specifically with lipids and proteins in aqueous solution where it promotes peroxidations. Due to its hydrophobicity and toxicity, heme has to be transported to its target proteins by different mechanisms, e.g. transport by transmembrane proteins, heme binding proteins and heme chaperones.
We identified *E. coli* HemW as a heme binding protein. To characterize the heme-binding *E. coli* HemW we overproduced, anaerobically purified HemW and upon heme supplementation the protein binds heme covalently and dimerizes. First EPR spectra of *E. coli* HemW incubated with heme revealed a spectrum typical of an oxidized [4Fe-4S]$^{3+}$ cluster indicating electron transfer from the cluster to heme. Supplementation of HemW with an EPR active Fe-Corrole revealed a 5x- and to a lesser extent 6x-coordinated heme, the latter being an unusual form of coordination for heme. Furthermore, HemW was able to restore the activity of heme-depleted nitrate reductase.
For further characterization of heme binding different spectroscopic methods (Raman resonance, Mössbauer, MCD) and binding constant using ITC will be used. The involved amino acid residues, the function of the iron sulphur cluster and SAM will be determined. Furthermore, the elucidation of the crystal structure will add to our understanding.
To verify that HemW is truly a heme chaperone, heme-depleted proteins such as e.g. Cytochrome *bd* oxidase will be tested for heme transfer.

FTP022

Potential of aerobic and anaerobic biodegradation of three benzotriazoles by pure and mixed cultures

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The wide-spread pollutants benzotriazole (BTri) and its two derivatives 4-methyl-benzotriazole (4-TTri) and 5-methyl-benzotriazole (5-TTri) were studied here to elucidate their aerobic and anaerobic biodegradation potential.
Aerobic mixed and pure cultures were derived from benzotriazole acclimatized activated sludge that was taken from stage 1 of a conventional two-stage activated sludge plant. Isolated on agar-plates with specific media, these cultures were used to inoculate various batch reactors (ranging from 1 to 250 mL in volume) with different selective media. These reactors, containing pure cultures as well as mixed cultures, were spiked with varying high concentrations of the three benzotriazoles (from 1.0 to 1000.0 mg/L) to evaluate the maximum concentration being biodegraded. Biodegradation was analyzed preliminarily by a novel 'quick and dirty' UV-absorption measurement technique as well as by GC-MS/MS for exact concentrations determination. It was shown that the mixed culture setups proved efficient in aerobic biodegradation of 5-TTri, at concentrations up to 50 mg/L, within 8 days if acclimatized. Biodegradation of BTri up to 30 mg/L occurred only within a longer time period of 21 days, even if acclimatized. Biodegradation capabilities of pure cultures have yet to be tested.
Anaerobic experiments were conducted up to now solely with mixed cultures derived from digested sludge (from the digester of the above mentioned wastewater treatment plant) to evaluate possible anaerobic biodegradation of the three benzotriazoles. Given 20 mg/L benzotriazoles and either nitrate, sulfate, or the humic acids model compound anthraquinone-2,6-disulfonate as terminal electron acceptors, the anaerobic biodegradation potential was compared to setups containing no additional electron acceptor. Biodegradation of benzotriazoles in the presence of electron acceptors seems likely, but has to be conclusively proved in further experiments. Without additional electron acceptors, no biodegradation has occurred yet. Ongoing research has to be done to verify this aspect.

FTP023

Bioremediation potential of bacteria isolated from petroleum-polluted soil in the Arabian Gulf region

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The Arabian Gulf region has witnessed the worst oil spill in history during the 1991 Gulf war. Although this environmental catastrophe occurred more than two decades ago, its impact on both aquatic and terrestrial ecosystems is still apparent. Bioremediation has been investigated as a cost-effective and eco-friendly approach for treatment of petroleum-polluted sites. In this study, several bacterial strains were isolated from petroleum-polluted soil via enrichment in chemically defined medium containing 2% Arabian light crude oil as the sole carbon and energy source. Based on 16S rDNA gene sequences and phylogenetic analyses, the isolates could be affiliated as strains of *Achromobacter*, *Bacillus*, *Pseudomonas*, and *Microbacterium* spp. All the isolated bacteria grew with 5 % crude oil as a sole carbon and energy source in chemically defined medium at 30℃. Two of the isolated bacteria grew with up to 20 % crude oil in chemically defined medium. Some isolates grew with crude oil at temperatures up to 55℃. Gravimetric analysis of residual oil after 5 weeks of incubation at 30℃ revealed 20 to 36% loss of the original oil amount added to the shake-flask cultures. Biodegradation of crude oil in all experiments was confirmed by Gas Chromatography/Mass Spectrometry (GC/MS). All the tested isolates emulsified crude oil in the culture medium within 1 to 7 days of incubation. Thus, indicating production of biosurfactants/bioemulsifiers in cell-free culture supernatants which was confirmed by the oil displacement assay.
The multiple phenotypes of some of the isolated bacteria (thermophilic, degradation of crude oil, biosurfactants/bioemulsifiers production) make them promising candidates for bioremediation of petroleum-polluted soil.

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FTP024

New insides into the naphthalene carboxylase in N47

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The understanding of biochemical processes underlying the anaerobic degradation of non-substituted aromatic compounds are still at their infancy. Metabolite analyses have indicated that for the model compound naphthalene, carboxylation rather than methylation was the first reaction activating such chemically stable molecule. We have recently brought biochemical evidence confirming the carboxylation reaction in the sulfate-reducing culture N47. Naphthalene carboxylase converts naphthalene and ^{13}C-labelled bicarbonate to 2-[carboxyl-^{13}C]naphthoic acid at a rate of 0.12 nmol min^{-1} mg^{-1} of protein in crude cell extracts of N47. Additionally, it exhibits an isotopic exchange capability indicating the formation of free reversible intermediates. Furthermore, the enzyme is reversible and catabolizes the formation of naphthalene from 2-naphthoic acid in the presence of only N47 crude cell extract. New lines of evidence from various experimental sets suggest that naphthalene carboxylase is a membrane-associated enzyme.

FTP025

Improvement of transformation efficiency and characteri-zation of a Δ*nth* mutant in *Staphylococcus carnosus* TM300

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Endonuclease III is described to cleave phosphodiester bonds at apurinic or apyrimidinic sites in the DNA and play crucial roles in some steps during base excision repair of DNA. Some of the nucleases limit the DNA uptake efficiency in bacteria by degrading foreign DNA. In the genome of *Staphylococcus carnosus* TM300 (*S. carnosus* TM300) were annotated several genes with similarity to nucleases. Among the genes was one that was homologous to the endonuclease III gene, *nth* (sca_1086).
In this study we generated a *nth* deletion mutant in *S. carnosus* TM300 and studied its functions by comparative phenotypic analysis. Nuclease activity in cell crude extracts was assayed by using undigested λ-DNA as a substrate. Indeed, in the Δ*nth* mutant degradation of λ-DNA was significantly decreased compared to wild type (WT) *S. carnosus* TM300. As the *S. carnosus nth* might also be involved in DNA repair the susceptibility to various mutagens was investigated. Two differently acting mutagenes were tested: Hydrogen peroxide (H_2O_2) and mitomycin. While the minimal inhibition concentration (MIC) of mitomycin was unchanged, the survival rate of the Δ*nth* mutant to H_2O_2 or streptomycin stress was clearly impaired. The mutagenic survival assay was carried out by the classical Ames-test. It turned out that the frequency of spontainous streptomycin and mitomycin resistant mutants was approximately three-fold increased in the Δ*nth* mutant, suggesting that the DNA repair activity was impaired. Finally, the transformation efficiency of Δ*nth* and WT strain was investigated and it was found that Δ*nth* showed three-fold higher transformation efficiency than the WT. These results show that the putative endonuclease III in *S. carnosus* TM300 has important functions in both, DNA repair and degradation of exogenic DNA.

FTP026

Enumeration and identification of resistant bacteria in municipal and hospital effluents

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Resistant bacteria in water environments are an increasing concern. The question has been raised about the clinical impact of environmental resistance and currently little is known about the dynamics of resistant bacteria and genes encoding resistance in sewage treatment plants.
We compared different effluents by selective, non-selective, and enrichment culture (Table). The number of at all cultivable enterobacteria, extended spectrum ß-lactamase (ESBL) and carbapenemase producing bacteria (CRB), and vancomycin resistant enterococci (VRE) were determined. The water samples were taken from a (i) central municipal sewage treatment plant with incoming water of about 24.6 Mio m^3/yr (CME_IN)and respective outflow (CME_OUT), (ii) a defined urban effluent (DUE) of solely human origin, (iii) and an effluent (420.000 m^3/yr) of a 1,200-bed hospital (HOE). Samples were taken in summer and autumn and more than 800 cultured bacteria were identified by MALDI-TOF MS fingerprinting with Maldi *Biotyper* (Bruker, Germany). Respective resistances were confirmed by Etest®(Oxoid), ESBL detection disks (MAST) or carbapenemase identification disks (Rosc

TABLE: Bacterial counts from summer and autumn effluent sampling revealed by direct plating on different agar media

	CME_IN	DUE	HOE	CME_OUT
Summer				
CASO 37°C	6.73E+05	n.d.	8.59E+05	1.02E+03
chromagarESBL	2.65E+03	n.d.	1.9+03	NOT even after
MacConkey + Ertapenem	4.35E+02	n.d.	1,35E+03	NOT even after
chromagarVRE	1.6E+03	n.d.	3.6E+03	1.0E+01
Autumn				
CASO 37°C	3.15E+07	6.4E+05	1.33E+06	6.5E+02
chromagarESBL	2.53+03	2.23E+03	2.66E+04	NOT even after
MacConkey + Ertapenem	2.45E+03	Only after enrichment	3.95E+03	NOT even after
chromagarVRE	1.45E+03	Only after enrichment	5.8E+03	NOT even after

n.d. not done

The CME_IN bacterial counts in autumn are 100fold higher than in summer. The DUE bacterial counts are lower in comparison to CME_IN and HOE. Of note is the peak of ESBL discharge in HOE in summer and the evidence that in DUE of solely human origin VRE are less present than in CME_IN containing other environmental sewage and agricultural waste water also of animal origin.
Enrichment culture of CME_OUT in summer as well as in autumn revealed several *E. coli* (n = 100) and *K. pneumoniae* (10) isolates but none of these isolates could be determined as ESBL. This is most probably due to non-selective conditions during the sewage treatment process resulting in loss of plasmids.

FTP027

Functional dissection of the Elongator subunit gene *ELP2/KTI3* from yeast

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Growth of *Saccharomyces cerevisiae* cells can be inhibited by zymocin, a tRNase ribotoxin produced from the dairy yeast *Kluyveromyces lactis*. For zymocin to target and cleave anticodons of several tRNA species, uridine bases in their wobble position (U34) need to be chemically altered in a fashion that requires the U34 modification function of the Elongator protein complex. Hence, U34 modification defects typical of Elongator mutants do efficiently protect against the tRNase attack of zymocin and cause the *killer toxin insensitivity* (*kti*) phenotype.
Here, we show that inactivation of *ELP2/KTI3*, the gene coding for the second largest subunit of the Elongator complex, causes phenotypes typical of other Elongator mutants and that the *ELP2* gene contributes to tRNA *nonsense* (*SUP4*) suppression and read-through of *ochre* mutations in reporter genes as diverse as *ade2-1* or *can1-100*. Our data, therefore, suggest the Elongator complex and its Elp2 subunit operate in proper tRNA functioning during the mRNA translation process. Interestingly, these tRNA related roles of Elp2 depend on C-terminal WD40 domains which, when mutated or truncated, elicit Elongator-minus phenotypes including zymocin resistance and abolish the ability of the Elongator complex to interact with other protein partners. This reinforces previous data showing that the physical contact between Elongator and additional interactors is required for Elongator function. Intriguingly, the Elp2 WD40 domains, which are conserved among other eukaryal homologs of the yeast Elp2 protein, appear to be engaged in protein protein interactions without affecting the integrity of the Elongator complex and/or assembly of holo-Elongator. This implies that by recruiting additional interaction partners to the Elongator complex, the Elp2 subunit may contribute to cofactor signalling and ultimately, be involved in the regulation of Elongator activities including the U34 modification function.

FTP028

Phospho-regulation of the budding yeast elongator subunit 1 (Elp1p) is required for normal interactions with Kti12/Hrr25

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Elongator is a six-subunit complex (Elp1-Elp6) that has roles in both transcription and translation. In the nucleus, Elongator interacts with RNA polymerase II and Elp3-mediated histone-acetylation is thought to be required for efficient transcription elongation. In the cytoplasm, Elongator is required for wobble uridine modification in tRNA anticodons to maintain efficient codon-anticodon pairing during mRNA translation. In this study, we set out to investigate yeast Elongator phosphorylation and its impact on Elongator function. Using mass spectrometry on purified Elongator complex, we have identified 17 phosphorylation sites on Elongator subunit 1 (Elp1). *In vitro* phosphorylation screening using a library of 120 yeast kinases has enabled us to link the Hrr25 kinase to the phosphorylation of one of these sites. Further phenotypic characterization of *elp1* phosphoablative and phosphomimetic substitution mutants suggested that at least 4 more phosphosites, including the one phosphorylated by Hrr25, is needed for normal interaction between the Elongator complex and Kti12, a protein known to interact with Elongator and to recruit Hrr25 to the Elongator complex. In summary, our study depicts a model for phosphorylation-dependent regulation of Elongator function that relies on appropriate interaction between Kti12 and the Elongator complex.

FTP029

Isolation of methanotrophic bacteria from termite gut

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Methanotrophs possess the unique ability to utilize methane as sole source of carbon and energy. The first step and key reaction of methane oxidation is catalyzed by methane monooxygenases (MMO), a characteristic enzyme of methanotrophs. One specific gene, encoding the β-subunit of the MMO holoenzyme, *pmoA*, is highly conserved and was used in this study to detect methanotrophic bacteria in the termite gut (Dedysh Svetlana *et al*. (2011); Methods in Enzymology; Vol. 495; pp.31-44). From the intestine of *Hodotermes mossambicus*, a gram-negative, aerobic, methane-oxidizing bacterium was isolated. Based on 16S rDNA gene sequence similarity, this strain was identified as a member of the family Methylocystaceae, belonging to the genus *Methylocystis*. The velocity of methane utilization was determined to be 1,62 pmol/cell·96h by gas chromatographic analysis. Species of this genus are type II methanotrophs and have been isolated from a multitude of environments. Characteristic features of this group are, inter alia, the serine pathway and membranes aligned with the cell periphery (Lindner Angela *et al*. (2007); Int. J. Syst. Evol. Micro.: Vol. 57; pp.1891-

1900). This is the first report known so far that demonstrates the presence of methanotrophic bacteria within termite gut microflora.

FTP030

Utilisation of proteolytically active lactobacilli for liberation of bioactive peptides from milk proteins

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Lactic acid bacteria (LAB) are of great interest because of their expansive application for production of various dairy products. As a consequence of several amino acid auxotrophies, LAB depend on exogen nitrogen sources such as caseins (α_{S1}-, α_{S2}-, β- and κ-casein) -which are the major milk proteins- as well as on an efficient proteolytic system providing amino acids for synthesis of endogenous proteins.
The aim of our study was to isolate, identify and characterise proteolytically active LAB for subsequent deployment for milk protein hydrolysis and liberation of bioactive peptides. In consideration of their proteolytic activities, we found primarly thermophilic lactobacilli, which exhibited highest proteolytic and caseinolytic activities among all LAB genera. Thereafter, we performed 16S-rDNA sequencing for identification and detected *L. delbrueckii* subsp. *delbrueckii* habouring highest proteolytic activity. For characterisation of caseinolytic behaviour of *L. delbrueckii,* specificity of casein degradation was studied with whole cell suspensions. The results indicated that *L. delbrueckii* preferentially degrades α- and β-casein, whereas for κ-casein almost no hydrolysis occurred. Furthermore, acidification curves, maximum acidification rates as well as cell growth in 10% skim milk were investigated in comparison to other proteolytically active strains. We found no correlation between proteolytic activity, acidification ability and cell growth, respectively.
LAB possess several peptide transport systems which may lower the (bioactive) peptide yield during casein hydrolysis. Hence, we successfully released proteolytic activity by using two different buffer systems and examined the cell free supernatants regarding to enzyme activity and protein profiles (SDS-PAGE). For release of bioactive peptides from milk, we applied cell suspensions to 10% skim milk. Released peptides were analysed by HPLC and pooled fractions will be tested for bioactivity (e.g. anti-oxidative).

FTP031

ZupT, a member of the ZIP family of zinc/iron transporters in *Cupriavidus metallidurans*

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The well-studied metal-resistant bacterium *Cupriavidus metallidurans* harbors a network of metal efflux systems with overlapping substrate specificities, which allows survival in highly heavy metal-polluted environments. Counterparts of these efflux systems are the primary and secondary metal uptake systems. Interaction of import and export reactions create a flow equilibrium of the cytoplasmic and periplasmic metal concentrations, which are kind of a "backbone" of cellular metal ion homeostasis. The central uptake system for Zn(II) and other ions in *C. metallidurans* is the ZIP (ZRT/IRT protein family) ZupT (2). Members of the ZIP protein family are ubiquitous, occurring in all kinds of organisms from bacteria to man, and play a key role in zinc transport. Similar to the first characterized bacterial ZIP protein from *E. coli* (1), ZupT from *C. metallidurans* contains 8 predicted transmembrane a-helices. The protein from *C. metallidurans*, however, possesses large histidine-rich loops that are not present in the enterobacterial transporter. To investigate the substrate specificity and kinetic parameters for metal transport, *zupT* from *C. metallidurans* was cloned into a pET28 derivate to add a N-terminal *His-tag* and expressed in an *E. coli* Rosetta strain. The expression of ZupT had a toxic effect on growth of *E. coli* and resulted in a low growth yield of the respective strain. Nevertheless, ZupT assembled into the membrane and could be detected due to its *His-tag*. ZupT could also be solubilized with sodium dodecyl sulfate (SDS) from the membrane, but not with other detergents tested.

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FTP032

Identification of Amino Acids of the PHB Binding Domain in PhaZ7 Depolymerase of *Paucimonas lemoignei*

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PhaZ7 depolymerase is the only known extracellular depolymerase that has been described as being active towards amorphous PHB (nPHB) [1]. The structure of PhaZ7 was solved first at 1.9 Å [2] and recently at 1.4 Å [3]. The active site of PhaZ7 is buried, so conformational changes must take place upon substrate binding. Comparison with other α/β hydrolases revealed that the structure of PhaZ7 is very similar to *Bacillus subtilis* lipase A except for an additional domain at one side of the molecule that is absent in LipA. Interestingly, this additional part of the enzyme is highly enriched in tyrosine and other hydrophobic residues. We suggest that this additional part could be responsible for interaction of the enzyme with the hydrophobic polymer. To find experimental evidence for this assumption we performed site-directed mutagenesis of selected positions in PhaZ7 and investigated the effect of the mutation on activity and polymer binding ability of PhaZ7. Our results showed that mutations of Y105, Y176, Y189, Y190 and W207 to alanine or glutamate resulted in reduced nPHB depolymerase activity and in an occurrence of a lag-phase at the beginning of the depolymerase reaction. The results of the binding assay of PhaZ7 with nPHB showed that Y105, Y176, Y189, Y190 and W207 mutein have reduced binding ability and verified that Y105, Y176, Y189, Y190 and W207 are essential for efficient PHB binding. Recently, the crystal structure of inactive PhaZ7 S136A mutein with bound 3-hydroxybutyrate (3-HB) trimer was also determined. It showed that 3-HB trimer is bound to a groove surrounded by Y105, Y176, Y189 and Y190. This result is consistent with our mutagenesis results. Interestingly, the superposition of free und trimer-bound PhaZ7 SerAla136A showed that both structures slightly differ from each other. The main changes were in the 280-295 and 248-251 region. The loop 280-295 was missing in the bound structure, suggesting some flexibility of these regions and their possible involvement in nPHB granules binding. Hence, the present results confirmed the involvement of Y105, Y176, Y189, and Y190 on PHB degradation.

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FTP033

Detection and analysis of biogenic sulfuric acid corrosion in wastewater systems

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Biogenic sulfuric acid corrosion (BSA) is one of the most serious and costly problems affecting the world´s sewerage infrastructure (e.g. concrete sewer pipes) and wastewater treatment (e.g. digesters). A complex microbial ecosystem, comprising sulfate reducing and sulfur oxidizing bacteria (SRB and SOB, respectively), is involved in the BSA process. The bacterial activity in the wastewater systems creates a sulfur cycle which can lead to bacterial formation of sulfuric acid (H_2SO_4) and consequently to corrosion of concrete. 20% of the total damage of concrete structures in sewer systems seems to be caused by BSA leading to global repair costs of several billions of dollars per year. Besides optical checks and acid analysis, currently, no precise test procedure is available for the detection of BSA attacks. Therefore, the aim of this research project (funded by AiF Projekt GmbH) is the development of a standardized biochemical test system for the accurate determination of the BSA potential in wastewater systems, especially in digesters. As a result, quantified information shall be gained about the extent of damage so that further evaluations about the structure stability can be made.
This test system will include (i) detection and quantification of SRB and SOB in the digested sludge and biofilm growing on the concrete surface, respectively and (ii) determination of the concrete corrosion potential carried out in specific simulation chambers inoculated with SRB and SOB and concrete specimens. A combination of different conventional microbiological as well as molecular-biological techniques such as polymerase-chain-reaction combined with denaturing gradient gel electrophoresis (PCR-DGGE), sequencing and phylogenetic sequence analysis, fluorescence *in situ* hybridization (FISH), and quantitative real

time PCR (qPCR) will be applied to detect, identify and quantify the relevant bacteria involved in the BSA process.
The test procedure would be a novel method to identify a BSA attack in wastewater systems both precisely and quantitatively. It would have an enormous economic impact worldwide, since it would provide the opportunity to discover BSA sufficiently early thusly preventing expensive modernization costs.

FTP034

Towards the isolation and identification of antimicrobial secondary metabolites from *Rhododendron*

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The Rhododendronpark Bremen hosts the World's second largest collection of *Rhododendron* species and thus might serve as an excellent resource for genetic diversity of this wooden plant genus. More than 550 different *Rhododendron* species and more than 3.200 hybrids are grown in this unique park setting. For centuries herbal medicine in the countries of origin of *Rhododendron* had taken advantage of the wound-healing, inflammation-suppressing, or generally health-promoting effects of extracts obtained from *Rhododendron*. However, these effects have never been directly associated with specific compounds and thus might have resulted from synergistically acting mixtures of compounds. Herein, an interdisciplinary approach was used to find out whether and how specific *Rhododendron* secondary metabolites might lead to novel natural product models for medicinal use. thus far, more than 160 different *Rhododendron* species were tested for their phylogenetic relatedness using a set of six different marker genes. The extracts of all species were assayed for anti-bacterial effects towards gram-positive and gram-negative test organisms, respectively. Effective extracts were further tested in various multidrug efflux pump mutants of different enterobacteria and pseudomonads. Subsequently, these extracts were analyzed in terms of their toxicity in cultures of intestinal epithelial cells as well as keratinocytes. Finally, extracts of the most promissing *Rhododendron* species were fractionated using tandem LC-MS. Fractions were re-assayed in bio-tests and subsequently analyzed in terms of chemical purity and structure using ion trap analyses. Preliminary results of this approach indicated that there are at least two different antimicrobial compounds obtainable from phylogenetically distant *Rhododendron* species. The plant extracts had variable effects towards different mammalian cell cultures with intestinal cells being generally more susceptible than keratinocytes.

FTP035

Investigating effects on carbon flow during biogas formation

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As partner of the network BioPara, our aim is to investigate biochemical processes which have negative or positive effects on the carbon flow during biogas formation. In high concentration, the organic acids acetate and butyrate can have a negative effect on the efficiency of biogas plants. High acidification results in a decrease of the methane production. In our study, we focused on the mechanisms of acidogenesis, which is a key process during biogas formation. To identify possible bottle necks, we addressed the key enzymes of acidification such as acetate kinase, butyrate kinase and butyryl-CoA:acetate-CoA transferase. As a first step, enzyme activity assays were established using crude cell extract from pure cultures (e.g. *Clostridium acetobutylicum*) and samples from metaproteome extract of labscale biogas reactor operated at high loading rates. Additionally, acetogenic bacteria were identified using the functional genes of the butyrate kinase (*buk*) and butyryl-CoA:acetate-CoA transferase (*but*) [Louis et al., 2004; Louis and Flint, 2007].
The carbon flow to convert methane to biogas could be positively affected by microbial community engineering. Therefore, we investigated the potential of cellulolytic clostridia to enhance the degradation rate of fibre rich substrates such as maize or grass silage. Potential candidates were *C. populeti*, *C. phytofermentans*, *C. cellulovorans*, and *C. cellulolyticum*. These bacteria were grown in cellulose-containing media and 10^9 cells ml^{-1} inoculated into biogas reactor content sthat had ceased biogas production. Afterwards, additional biogas formation was measured in comparison to a control.

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FTP036

Biosynthesis of the antimycoplasma agent micacocidin

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In a recent study we identified the biosynthesis gene cluster for the production of the antimycoplasma agent micacocidin in the bacterium *Ralstonia solanacearum* GMI1000 (1). The corresponding locus includes genes for two distinct classes of biosynthetic enzymes, namely polyketide synthases and nonribosomal peptide synthetases. Notwithstanding the modular logic of these multifunctional enzymes (2), it was not possible to deduce the structure of micacocidin from an analysis of its assembly line by existing biosynthetic models. In particular, the origin of the distinctive 6-pentylsalicylate moiety (PSA) in micacocidin was puzzling. A feeding study with stable isotope precursors as well as biochemical characterization of selected catalytic domains revealed an unexpected biosynthetic pathway featuring hallmarks of fungal polyketide assembly. Here, we report the reconstitution of essential steps leading to PSA formation and propose a mechanistic model that is consistent with our findings.

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FTP037

Improved acetate production of *Aceto-bacterium woodii* grown on CO_2/H_2

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Acetate is usually produced chemically by the carbonylation of methanol. Acetogenic bacteria are also able to produce acetate via the Wood-Ljungdahl pathway. Heterologous overexpression of the genes *pta* (phosphotransacetylase) and *ack* (acetate kinase) or the genes coding for formyl-THF-synthetase, methenyl-THF-cyclohydrolase, methylene-THF-dehydrogenase, and methylene-THF-reductase of the Wood-Ljungdahl pathway of *Clostridium ljungdahlii* in *A. woodii* led to a higher acetate production of the recombinant cells grown on CO_2/H_2. In this study, we developed a transformation protocol for *A. woodii* and were able to increase the maximal acetate production by overexpression of the *pta-ack* operon and by overexpression of the genes encoding for the formyl-THF-synthetase, methenyl-THF-cyclohydrolase, methylene-THF-dehydrogenase, and the methylene-THF-reductase in *A. woodii*. Fermentation experiments in a stirred-tank reactor on the liter-scale using a CO_2/H_2 gas mixture with controlled pH (7.0) showed a maximal acetate production of 51 g/l after 3.7 days with the recombinant strains.

FTP038

Symbiont transmission in the gutless marine worm Olavius algarvensis

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The gutless oligochaete Olavius algarvensis, from the island of Elba in the Mediterranean Sea, lives in an obligate symbiosis with a consortium of sulphide-oxidizing gammaproteobacteria, sulphate-reducing deltaproteo-

bacteria and a spirochaete. The complete dependency of the host on its endosymbionts has led to the reduction of its digestive and excretory system. To better understand the evolutionary processes that have led to the establishment of this symbiosis we are currently examining how symbionts are transmitted from one generation to the next. In contrast to other oligochaetes, eggs of gutless oligochaetes initially lack a rigid cocoon. This means that when the worms deposit their eggs in the sediment, free-living symbiont stages, provided these exist, could be horizontally transmitted from the environment to the egg. Vertical transmission during the egg laying process through smearing of symbionts from the worm's genital pads to the egg is another possibility, and has been described in a gutless oligochaete from Bermuda. These two transmission modes are not mutually exclusive and we are therefore examining both possibilities in O. algarvensis.
Fluorescence in situ hybridization (FISH) analyses of adult worms revealed that neither the unfertilized eggs nor the sperm contained symbionts. This indicates that the transmission of symbionts must occur at a later stage of host development, as they are neither passed through the male nor female germ lines directly. FISH with general gammaproteobacterial and deltaproteobacterial probes showed that small juveniles under 1 mm length were already colonized by symbionts. The time frame for transmission of symbionts therefore must occur between egg deposition and hatching of the juvenile. With this ongoing study we will investigate which of the five symbiotic bacterial species are transmitted during oviposition by smearing from the parent worm and which, if any, are taken up from the environment after deposition of the egg.

FTP039
Sympatric co-speciation in a shallow-water chemo-synthetic symbiosis

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Gutless oligochaetes are small marine worms that live in an obligate symbiosis with up to six bacterial endosymbionts and occur worldwide in warm to temperate shallow-water sediments. These worms lack both a digestive and an excretory system and are dependent on their symbionts for gaining nutrition and the recycling of their waste products. All gutless oligochaetes harbor a primary sulfur-oxidizing symbiont, while the composition of the secondary symbionts varies in different host species.
In this study, we examined the genetic variability of the symbiosis in the gutless oligochaete *Olavius algarvensis* from Mediterranean shallow-water sediments. Sequencing of the mitochondrial cytochrome c oxidase (COI) gene of 50 *Olavius algarvensis* individuals from the Sant' Andrea Bay on Elba revealed two haplotypes, indicating that two genetically distinct host populations co-occur at this site. Analyses of the 16S rRNA gene of the sulfur-oxidizing primary symbiont of these hosts revealed a similar separation into two distinct phylotypes, that is, each host haplotype had a distinct and specific symbiont phylotype. This suggests that the primary symbionts of *O. algarvensis* are co-evolving with their host in sympatric speciation. In contrast, the 16S rRNA genes of the three other symbionts of *O. algarvensis* were identical in the two host populations. Co-speciation between symbionts and their hosts is common in associations with vertical transmission of the symbiont from one host generation to the next. Indeed, our preliminary studies of symbiont transmission in *O. algarvensis* indicate that the primary symbiont is transmitted vertically, whereas the secondary symbionts are transmitted in a mixed mode of vertical and horizontal transmission, possibly with frequent host-switching events taking place between the two host populations. We are currently sequencing the genomes of the primary and secondary symbionts from the two co-occurring *O. algarvensis* host populations to better understand the evolutionary relationships between these hosts and their symbionts, and the processes that drive their diversification.

FTP040
Aconitase (AcnA) from *Streptomyces viridochromogenes* TÜ494 as a moonlighting protein: the analysis of its catalytic and regulatory role.

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Protein moonlighting is a phenomenon by which a protein can perform more than one function. Aconitases in many organisms play a dual role: they serve as primary metabolism enzymes in the tricarboxylic acide cycle (TCA) and as regulators of iron metabolism and oxidative stress response. It was shown that inactivation of the primary metabolic aconitase AcnA in *Streptomyces viridochromogenes* TÜ494, the producer of the herbicide antibiotic phosphinothricin tripeptide (PTT), leads to strong defects in physiological and morphological differentiation. AcnA is not only an enzyme but also belongs to Iron Regulatory Proteins and posses characteristic Iron Responsive Element (IRE)-binding motif what reveals an additional function of this protein in regulation of iron metabolism and oxidative stress as it is described for Iron Responsive Proteins (IRPs). *In silico* analysis of the *S. viridochromogenes* genome revealed several IRE-like structures. One structure is located upstream of *recA*, which is involved in the bacterial SOS response, and another one was identified upstream of *ftsZ*, which is required for the onset of sporulation in streptomycetes. The functionality of the IRE structures was proven using gel shift assays. Furthermore, RecA was shown to be up-regulated on posttranscriptional level under oxidative stress conditions in the wild-type strain but not in the *acnA* mutant, supporting the idea of a regulatory role of AcnA in oxidative stress response.

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Heinzelmann E, Kienzlen G, Kaspar S, Recktenwald J, Wohlleben W, Schwartz D. 2001 The phosphinomethylmalate isomerase gene pmi, encoding an aconitase-like enzyme, is involved in the synthesis of phosphinothricin tripeptide in *Streptomyces viridochromogenes*.

FTP041
PqsL of *Pseudomonas aeruginosa*, an enzyme involved in the biosynthesis of 2-alkyl-4-hydroxyquinoline-*N*-oxides

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2-Alkyl-4(1*H*)-quinolones (AQs) and 2-alkyl-4-hydroxyquinoline-*N*-oxides (AQNOs) have originally been identified as secondary metabolites of fluorescent pseudomonads, exhibiting antimicrobial activity primarily towards Gram-positive bacteria (reviewed in [1]). AQNOs not only serve as antimicrobials, acting as inhibitors of respiratory electron transport through the cytochrome bc_1 complex, but can also elicit physiological responses at subinhibitory levels [2].
Biosynthesis of AQs in *Pseudomonas aeruginosa* requires the *pqsABCD* genes [3]. Formation of AQNOs additionally involves *pqsL* [4], which codes for a putative flavin-dependent monooxygenase. However, the reaction catalyzed by PqsL has not been identified yet.
Biotransformation experiments using recombinant *P. putida* KT2440 strains that co-express *pqsL* and combinations of *pqsA, pqsB, pqsC* and *pqsD* from *P. aeruginosa* PAO1 confirmed that all these *pqs* genes are required for AQNO synthesis from anthranilate and fatty acid precursors. Consistent with the proposal that AQs are not the direct precursors of AQNOs [5], *P. putida* [pME6032-*pqsL*] did not oxidize 2-heptyl-4(1*H*)-quinolone (HHQ) to the corresponding *N*-oxide.
PqsL protein was purified from a recombinant *E. coli* strain by means of an N-terminal StrepII affinity tag, and its interaction with potential substrates or substrate analogs was analyzed by fluorescence titration. Binding of HHQ to PqsL was not observed, however, several quinoline derivatives containing a tertiary ring nitrogen and a substituent at C-4 weakly interacted with the protein (e.g., a dissociation constant K_d of 72 μM was determined for the complex of PqsL with quinoline-4-carboxylic acid). On the other hand, the K_d value of about 8 μM of the complex with anthraniloyl-CoA, together with the high K_d of 175 μM observed for the ethylanthranilate-PqsL complex, suggested that PqsL has affinity for the CoA moiety. An activity assay will be developed to characterize the reaction catalyzed by PqsL.

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FTP042

Spatial distribution of microorganisms involved in N-cycle in different types of constructed wetlands

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This research explores the role of aerobic and anaerobic microbial processes in the removal of ammonium from contaminated groundwater in constructed wetlands (CWs) downstream of the chemical industrial area Leuna/ Germany.
While the anaerobic ammonium oxidizing (Anammox) bacteria have been found in many different natural environments there is still lack of knowledge in the role of these bacteria in CWs. Investigations are necessary to learn more about competition between anaerobic ammonium oxidizers and other groups of ammonia oxidizing bacteria in the ecology of various wetland systems.
Therefore, the focus of current research is: to explore spatial distribution in biofilms in CWs of Anammox bacteria as well as nitrifying bacteria and some other bacterial groups of N-cycle. For this aim, three different types of CWs were chosen: planted horizontal subsurface flow (HSSF-CW), unplanted HSSF CW, and floating plant root mat (FPRM). Samples of roots and gravel were taken at different flow distances from the inlet and at different depths of the systems.
DNA from biofilms at roots and gravel was extracted using FastDNA® Spin Kit For Soil (MP Biomedicals). At next steps Pyrosequencing and specific FISH probes in connections with confocal laser scanning microscopy will give information about structure and spatial distribution of the microbial nitrogen transforming community.
By combining data of molecular-biological techniques with data of $^{15}N/^{14}N$ variations in nitrogen compounds and with physico-chemical parameters (pH, rH, concentrations of N-compounds) more profound results about the nitrogen removal processes in CWs will be shown.

FTP043

Alternative per se specification and validation of real-time PCR assays for applied biotechnology based on testing algorithms derived from other scientific and technical research areas

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The implementation of molecular-biological based pathogen detection is a frequently and intensively discussed topic. Molecular-biological methods for food analysis comprise a detection chain consisting of sample preparation, target purification and a detection assay (qPCR). Given this systemic character systems theory provides basis for discussion of principles and application of testing methods derived from various scientific areas for specification and validation of pathogen detection. This talk describes the structure and strategy of a possible alternative approach for validation and specification of qPCR to accelerate its broad range implementation into routine diagnostics. The hypothesis is established that systems theory provides the basis for implementation of test systems, derived from other scientific or technical areas, to specification and validation of qPCR based pathogen detection, or alternatively as supplemental to existing international standards. The categorisation of black box and white box systems demonstrates a possible classification for pathogen detection methods. The resulting applicability of Physical-Modelling-Synthesis and System-Identification as used in software development and electrical engineering provides two strong instruments for validation of the complete molecular-biological detection process. Equivalence-Class-Formation and Boundary-Limit-Analysis, which is the underlying test principle for approvable application of System-Identification, both support specification of the qPCR assay building the core of a molecular-biological detection chain. This alternative approach is based on validation of the method per *se* and supports conventional comparative validation according to ISO 16410.

FTP044

Copper-sensing in *Escherichia coli* - Role of CusS

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Escherichia coli harbors three systems for copper detoxification on the chromosome. The cytoplasmic membrane localized, coppertransporting P-type ATPase CopA catalyses the energy dependent transport of Cu(I) out of the cytoplasm (1). The multicopper oxidase CueO detoxifies the periplasmic space from excess copper through oxidation of Cu(I) to Cu(II). Gene expression of *cueO* and *copA* is regulated by CueR, a Me rR-Type regulator, which detects increasing copper concentrations in the cytoplasm. Another periplasmic complex, CusCFBA, exports copper out of the cell. The two-component-system CusRS acts as a sensor for copper ions. CusS, the sensor histidine kinase, anchored in the cytoplasmic membrane is responsible for detection of copper and silver ions (2). After metal binding a phosphate-group is transferred from CusS to CusR, the appropriate response regulator, leading to DNA-binding at the *cop*-box followed by transcription of *cusCFBA* and *cusRS*.
Efflux of Cu(I) and Ag(I) ions by CusCBA out of the periplasm could be shown (1). Under anaerobic conditions the oxygen dependent multicopper oxidase CueO is inactive, so Cus is the most important system. Following the hypothesis, that Cus can detoxify the cytoplasm as well as the periplasm, we investigate possible copper binding sites of CusS. Several mutant-proteins were analysed and topological studies were done.

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FTP045

Characterization of a novel PQQ-dependent aldehyde-dehydrogenase isolated from *Shingomonas wittichii* RW1

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The α-proteobacterium *Sphingomonas wittichii* RW1 is prominent for its ability to degrade dioxins and related toxic substances (1). Bioinformatic analysis of the genome indicated that this organism may contain the largest number of pyrroloquinoline quinone-dependent dehydrogenases (PQQ-DHs) of all bacteria sequenced so far. Sequence analysis also showed that one of these genes (swit_4395) encodes an enzyme that belongs to the class of periplasmic soluble glucose/sorbosone dehydrogenases. This gene was fused to a pelB and a strep-tag coding region at the 5´and 3´ end, respectively, and was cloned into the broad-host range expression vector pBBR1p264 (2). The corresponding protein was then heterologously produced in *E. coli,* purified via Streptactin affinity chromatography and characterized. The protein Swit_4395 showed a distinct band in an SDS-PAGE at 41 kDa. Native PAGE in combination with activity staining indicated that it is active as a homohexadecamer and a homododecamer. Enzymatic assays displayed that Swit_4395 was an aldehyde dehydrogenase that showed the highest activity with medium-chain aldehydes (chain length C3-C5) and ketoaldehydes such as methyglyoxal and phenylglyoxal. Butyraldehyde was the best substrate with Vmax and Km values of 3300 U/mg and 10 mM, respectively. PQQ was identified as prosthetic group of the purified enzyme by spectroscopic methods. When the expression vector containing *swit*_4395 was electroporated into *Sphingomonas wittichii* RW1 homologous production of the recombinant protein was observed and the enzyme could be purified from the native host. In contrast to the wild type, the expression strain was able to grow on butanol when PQQ was added to the medium indicating that this enzyme is necessary for the utilization of medium chain alcohols.

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FTP046

Splicing activity of the *Picrophilus torridus* A-ATPase catalytic subunit A intein at native and foreign extein sequences

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Inteins are self-splicing protein elements that interrupt the peptide sequence of a host protein. They excise themselves from the precursor protein and ligate the flanking sequences with a native peptide bond in an autocatalytic process without the need of a cofactor. An intein with a partially conserved endonuclease motif is embedded within the A-ATPase catalytic subunit A from the thermoacidophilic archaeon *Picrophilus torridus*. To identify the smallest natural particular sequence context of this intein that allows for efficient protein splicing, single amino acid residues of the native extein sequences were consecutively deleted at the splice site junctions. Splicing activity was investigated in a heterologous precursor protein composed of a N-terminal GST-tag and a C-terminal His-tag fused to native amino acid residues of the A-ATPase catalytic subunit A. A set of 26 different precursor variants were generated and analyzed with regard to their splicing abilities using SDS-PAGE and Western blotting analysis. To prove the results obtained from the GST-intein-His models, the gene encoding the intein in combination with native flanking amino acids residues was introduced into several insertion sites in glycoside hydrolases. The identification of essential extein residues for this intein is a good starting point to generate optimal universal foreign-flanking extein sequences within artificial host proteins as well as to develop advanced intein reporter systems.

FTP047

Developing a detection system for the genus *Bacillus* opens up new insights into its phylogeny

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Hygiene monitoring is performed routinely within pharmaceutical clean rooms. Therefore, rapid identification of contaminating bacteria is fundamental to ensure germ-free or low-germ products. In this context, members of the spore-forming genus *Bacillus* are of particular importance since they require stronger disinfection procedures than others.
The development of a detection system for *Bacillus* species using real-time PCR is challenging as the genus represents a heterogeneous group comprising over 150 species. Additionally, re-classification within the family Bacillaceae occurs frequently causing a continuously changing number of *Bacillus* species. Due to complexity and heterogeneity, detection of the genus by a single primer and probe system is not feasible. Thus, our approach includes two steps: 1) assessing the species relevance and 2) generating subsets enabling their collective detection.
Firstly, hygiene monitoring-relevant species were selected from the whole *Bacillus* genus according to their growth requirements. Only aerobic, spore-forming species were considered as relevant for our application. In addition, members of the genus possessing extremophilic properties were excluded since they will not be able to grow under hygiene monitoring conditions (20-35 °C, pH 6-8, 0-1% NaCl).
Secondly, valid sequences of the relevant *Bacillus* species (>100) were used to build a phylogenetic tree, which allows the formation of subsets to achieve group-wise identification. Subsequently, sequence regions were determined to design appropriate primer-probe-systems. In consequence, newly isolated species belonging to one of the defined subsets can be covered by this detection system.
Our strategy facilitates the development of primer-probe-systems for a high number of species. We have elaborated an effort-reducing and application-oriented method that combines phylogenetic and physiological data raising a new perspective on *Bacillus* systematics.

FTP048

Biosensor Stability - Application of Hydroxyectoine in Carbon Nanotube-based Bioelectronics

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In contrast to the well-known compatible solute ectoine, its hydroxylated derivative (S,S-β-hydroxyectoine) is a good glass-former (vitrificant) and as such imployed by the halophilic *Halomonas elongata* for survival in the dry state. The importance of such organic glasses has been well documented for anhydrobiotic organisms like tardigrades, resurrection plants and others (usually with sugars like trehalose and sucrose). In the context of anhydrobiotic engineering trehalose and hydroxyectoine were likewise applied to stabilise desiccation-sensitive biomaterial [1-5].

As long-term biostabilisation is also one of the main challenges in biosensorics it seems logical to apply such compounds to accomplish enhanced shelf-life of biohybrid devices. Electroconductive carbon nanotubes (CNT) are typically used in bioelectronic sensors. As the delocalized π system of ectoines is known to interact with aromatic residues in binding proteins [6], a similar interaction with aromatic carbon nanotubes seemed likely. Hence, the glass-forming ability of hydroxyectoine in conjunction with a possible adsorption to the nanotubes appeared to be a promising strategy to improve the desiccation stability of such biosensors. To test the influence of ectoines, a CNT-based biosensor with immobilised glucose oxidase was successfully contructed. Electrochemical characterization was accomplished by cyclovoltammetric experiments and demonstrated improved electron transfer from glucose to the electrode, when prepared in the presence of hydroxyectoine (up to 50 %) In subsequent drying procedures hydroxyectoine also proved beneficial for electro-communication on this biohybrid interface.

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FTP049

Gold transformation by *Cupriavidus metallidurans*

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Cupriavidus metallidurans could be responsible for the formation of bacteriogenic secondary gold nanoparticles [1,2]. We investigated if gene clusters that are up-regulated after treatment with gold complexes might be involved in this process. The two indigenous plasmids of *C. metallidurans*, pMOL28 and pMOL30, which harbor several transition metal resistance determinants, are not involved in resistance to Au(I/III)-complexes and in the transformation to metallic Au(0) nanoparticles. Up-regulation of a *cupA-lacZ* fusion by the MerR-type regulator CupR with increasing Au(III) concentration indicates the presence of gold ions in the cytoplasm. A hypothesis that the *gig* (gold-induced genes) cluster detoxifies gold complexes by uptake and/or reduction of Au(III) to Au(I) or Au(0) reminiscent to mercury transformation by *mer* gene products was falsified. The deletion of ZupT and other secondary uptake systems for transition metal cations increased Au(III) sensitivity but had no influence on up-regulation of the *cupA-lacZ* fusion or metal content of the cells. Furthermore, we investigated the contribution of copper resistance systems to gold resistance. The *copABCD* determinant on chromosome 2, which encodes periplasmic copper resistance proteins, was required for full gold resistance in *C. metallidurans*.

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FTP050

Recombination events of the 2-micron plasmid and YEp-like recombinant plasmids in *Saccharomyces cerevisiae*

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The budding yeast *Saccharomyces cerevisiae* is one of the few eukaryotic organisms naturally wise harboring a plasmid, the 2-micron (2-µm) plasmid[1]. This multicopy plasmid is well studied and known to be present in the cells in two isomeric forms (A and B)[2], generated by intramolecular recombination during plasmid amplification.
Numerous advantages have generated an interest in yeast for the production of genetically engineered products, particularly proteins and pharmaceutical components[3]. Hybrid plasmids (e.g. YEp-like vectors) carrying heterologous expression blocks use the replication origin (*ori*) of the 2-μm plasmid for amplification and partitioning. These plasmids ensure increased levels of productivity due to their multicopy nature. In industrial production a major concern is plasmid instability in recombinant production strains, i.e. plasmids might get lost, sometimes at high frequency, as a result of the additional metabolic burden. Rearrangements of such plasmids with a concomitant loss of the respective expression blocks (or parts of it) have been observed as well.
YEp-like plasmids use *cis* (*ori*) and, likely, *trans* (FLP recombinase encoded in the 2-µm plasmid) acting elements for amplification, partitioning, and maintenance. The amplification process suggests interactions (*via* homologous recombination) between YEp-like plasmids and the 2-µm plasmid. Such events might support plasmid rearrangements. Southern blotting allows the visualization of a reproducible pattern, indicating the presence of additional forms of the 2-µm plasmid beyond the afore-mentioned A and B isomers. PCR based methods are currently being developed for identifying and, hopefully, quantifying molecular forms of such interactions.

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FTP051

Antibiotic permeation through membrane channels: Role of *Providencia stuartii* porin in β-lactam resistance

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The outer membrane of gram-negative bacteria contains a number of channel-forming hydrophilic proteins called porins. Such channels allow the diffusion of low molecular weight solutes across the outer membrane. Antibiotics are known to use the porin-channel pathway to cross the permeability barrier of bacteria. In this study we focused on a porin from *Providencia stuartii*, OmpPst1 and its interaction with antibiotics such as imipenem using high resolution electrophysiology. Analysis of the ion current fluctuations through OmpPst1 in presence of imipenem revealed kinetic parameters of antibiotic binding. In addition, the effect of divalent and trivalent cations on antibiotic affinity to the channel was investigated. Electrophysiology measurements are complemented by liposome swelling assays which determine the permeation of antibiotics through porins reconstituted into liposomes. To follow the exact translocation pathway, molecular modelling provided details on the interaction of the molecules with the channel surface and the position of affinity sites. Activity of antibiotics against bacteria was determined by microbiological assays which correlates with the results obtained from planar lipid bilayer measurements.A combination of techniques was used to conclude on antibiotic permeation and their efficiency against the bacterium.

FTP052

Antibacterial activities against *Salmonella gallinarum* and *Bordetella bronchiseptica* by some bacterial isolates

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According to the increasing demands of pork and chicken meat, the farming of pig and poultry rapidly becomes high density large scale system. Consequently, the incidence rates of chronic respiratory disease in pig by *Bordetella bronchiseptica* and fowl typhoid by *Salmonella gallinarum* increased globally, and these cause the significant economic damage to farming industry. To prevent these diseases a lot of antibiotics are used, but occurrence of resistant pathogens brings another problem. Therefore, simple, cost-effective and environment-friendly measures are necessary to control these pathogens, and microbial agents can be an alternative one. We isolated 372 bacterial strains from the samples collected from pig and poultry farms, slaughter house, soils from near farm, and pig nose. Strain TK3, TK4 and TK5 show the antibacterial activity against both pathogens, and TK3 was identified as *Bacillus amyloliquefaciens*, and TK4 and5 were identified as *Pseudomonas aeruginosa* by 16S rDNA sequence analysis. TK6 showing the antibacterial activity against *B. bronchiseptica* was identified as *Alcaligenes faecalis* subsp. *parafaecalis*. The mechanisms of antibacterial activity of these isolated bacteria were examined. Siderophore inhibits the growth of pathogenic organisms through the high affinity to iron which is necessary nutrient to pathogens. Strains TK3, 4, and 5 produced siderophore measured by CAS agar plate assay. Production radius of siderophore by TK3, 4 and 5 were 0.53, 1.48 and 1.21 cm, respectively after 14 days of incubation, and the concentration of siderophore were 1056.3, 705.9 and 1102.7 µM ml^{-1}, which are quite high level. Strain TK3 also secreted other antibacterial compound, rhamnolipid detected by rhamnolipid biosurfactant screening medium, and the production rates were different with the kind of carbon substrates. Some strains also produced antibacterial peptides including lipopeptides.

FTP053

Auxin response in tomato plant by indole acetic acid produced by *Acinetobacter guillouiae* SW5

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Some rhizobacteria are considered to promote plant growth through various direct or indirect mechanisms including the action of a typical phytohormone, indole acetic acid (IAA). However, functional genomic studies are needed to unravel the function and mechanism of IAA signaling in bacteria and during the different stages of microorganism-plant interactions. In this study, the expression of auxin response genes, *LeIAA1* and *LeIAA9* in tomato was examined during the interaction between plant growth promoting bacterium and tomato plant. *Acinetobacter guillouiae* SW5 isolated from rhizosphere of *Setaria viridis* produced 1.3 mg mg $protein^{-1}$ (118.1 mg l^{-1}) of IAA in brain heart broth medium in 2 days of incubation. When the bacteria-produced IAA and standard chemical IAA were treated for 30 minutes to one-week old tomato seedlings, the copy numbers of *LeIAA1* and *LeIAA9* increased up to 12 and 4 folds, respectively compared to that of the control at range from 10 to 100 µM of IAA. The copy numbers of *LeIAA1* and *LeIAA9* increased by only live cells of *A. guillouiae* SW5 and by larger bacterial inoculants. When the tomato root exudate was analyzed by HPLC, 3.75 ± 0.95 ng mg $tomato^{-1}$ of tryptophan was detected, which is an IAA precursor and could be used to produce IAA by *A. guillouiae* SW5. Subsequently, this IAA secreted by bacterium might increase the expression of the *LeIAA1* and *LeIAA9* genes in tomato. This is the first report showing that bacteria-produced IAA may affect the expression of IAA response genes in plant.

FTP054

The effect of *Aloe vera* L. extract leaf to the growth of *Candida albicans* fungus *in vitro*.

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The aim of this research was to know the ability of *Aloe vera* L. leaf extract inhibit the growth of *Candida albicans* fungus in vitro. *Aloe vera* L. is the plant that can cure some absorption problems that are caused by fungus. One of fungus pathogen causes absorption infection is *C. albicans*. This research was done in Microbiology Laboratory at Biology Departement of Mathematic and Natural Science Faculty, Syiah Kuala University. The research was done from April 2007 until May 2008. This research used Completely Randomized Design (CRD) method with five treatments and five repetitions of each treatment. The treatment includes Po = aquades 20 gl, P1 = the concentration of *Aloe vera* L. leaf extract 100%, P2 = the concentration of *Aloe vera* L. leaf extract 50%, and P3 = the concentration of *Aloe vera* L. leaf extract 25%. 20 ul *Aloe vera* L. extract was given in each treatment. The parameter in this research was the diameter of inhibition

zone. The data was analyzed by Analysis of Variant (ANOVA) and continued with Leaf Significant Difference (LSD) test. The result showed that *Aloe vera* L. leaf extract can prevent the growth of *C. albicans*.The higher concentration of *Aloe vera* L. leaf extract, the higher ability to inhibit *C. albicans* became. Increasing the concentration of *Aloe vera* L. leaf extract will increase the diameter of inhibiton zone. Concentration 100%, 50% and 25% *Aloe vera* L. leaf extract have equal ability to inhibit *C. albicans* as nistatin antibiotic in concentration 0,50 mg, 0,24 mg dan 0,20 mg.

Keywords : *Aloe vera* L. , *Candida albicans*, inhibition zone.

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FTP055

Dynamics of biofilm formation during anaerobic digestion of organic waste

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The formation of anaerobic biofilms in biogas reactors improves the methane yield and the degradation rate of organic material during the biogas process (1). For the investigation of biofilm dynamics, polypropylene-discs were used, which served as biofilm carriers. Those discs were incubated in batch biogas reactors at high and low organic loading rates for different time periods. The dynamics of biofilm formation during anaerobic digestion were observed by epifluorescence-, light-, conventional scanning electron microscopy and environmental scanning electron microscopy. The resulting micrographs were analysed using the software ImageJ in terms of total cell numbers, thickness of biofilms, biofilm covered area and the area covered by extracellular polymeric substances (EPS).
In general, we found that the cell number within biofilms was in average five times higher compared to the numbers of cells in the fluid reactor content. Moreover, biofilm formation and structure mainly was correlated with the number of microorganisms present in the fluid reactor content and the organic loading. High organic loading rates (50 kg VS m^{-3}) and cell numbers contributed to a continuous biofilm layer. The thickness of the biofilm layer on the used discs ranged from 2 to 380 μm with an average of 70 μm. In contrast, at lower organic loading rates (15 kg VS m^{-3}) the biofilm formation was less pronounced. Microcolonies were detectable, which increased in frequency and size during anaerobic digestion. However, thickness and velocity of biofilm formation were not proportional to the organic loading. Independently from the organic loading rate, the microorganisms attached to the discs were completely covered by EPS within seven days of incubation. The maturation and maintenance of biofilms were related to the availability of substrate. The detachment of microorganisms within biofilms occurred simultaneously with the decrease of biogas formation. The mentioned positive effects of biofilms on the biogas process were most likely related to the higher cell numbers within the biofilms compared to the fluid reactor content.

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FTP056

A dual flagellar system in *S. putrefaciens* CN-32: impact of heterogeneity on effective swimming behavior

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Many bacterial species possess, in addition to single polar flagella, a second flagella system that could be involved in cellular motility under changing environmental conditions. Examples of bacterial species with secondary flagella are *Vibrio parahaemolyticus*, *Aeromonas hydrophila,* and *Shewanella putrefaciens* CN-32.
It has been observed that only a subpopulation of the model organism *S. putrefaciens* CN-32 truly expresses the secondary flagella, while other cells have only one or no flagella system. This might be part of a "bet-hedging" strategy of *S. putrefaciens* CN-32, as flagella systems are metabolically expensive both for their formation and maintenance. Therefore, it is hypothesized that one population attempts to establish bacterial growth in its original niche, saving energy, while other motile subpopulations can migrate and colonize new environments.
However, it is still unknown, how the expression of the lateral flagella is induced. On swim agar plate, a loss of motility is observed if the sole chemotaxis system in *S. putrefaciens* CN-32 is disturbed. It could be also shown that CheY, the chemotaxis response regulator, is exclusively interacting with the switch complex of the polar flagellum. Interestingly, the disruption of lateral flagellum assembly in a *cheY* mutant completely abolishes swimming motility through swim agar, whereas in liquid culture unidirectional movement of single cells is still intact. Thus, although the lateral flagella are not directly regulated by the chemotaxis system, there is strong evidence that lateral flagella are involved in improved directed swimming in planktonic conditions.

FTP057

BlueTox - a novel photosensitizer for efficient ROS-mediated cell killing

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Fluorescent proteins like the green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* generate reactive oxygen species (ROS) as a byproduct of their fluorescence activity [1]. The amount of generated ROS strongly depends on the structure of the respective fluorescent protein [2,3] with KillerRed representing an example for a high-level ROS-producing fluorescent protein which allows light-mediated cell killing [4]. In the current study, we investigated the light-dependent ROS-mediated toxicity of BlueTox, a genetically encoded photosensitizer that has been developed from a flavinmononucleotide-based fluorescent protein (FbFP) [5]. BlueTox binds FMN as fluorophor and emits green fluorescence (em^{max} 495 nm) after excitation with blue light (ex^{max} 450 nm). We analyzed the light-induced phototoxic effect of BlueTox in *E. coli* cells, as well as in eukaryotic hippocampal mouse tumor cells (HT22) in a time-resolved manner. The results of the *in vivo* investigations showed that BlueTox exerts a strong and fast inactivation of both pro- and eukaryotic cells thereby demonstrating its potential as a blue-light absorbing photosensitizer in cell- and microbiology.

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FTP058

Biometals content of cells during kingdoms of life, a general investigation by use of ICP-MS

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The cellular life depends on metal co-factors in a multitude of enzymes, such as urease, hyrogenase, superoxide dismutase or cytochrome oxidase. Biochemical approaches suggest that 10% of bacterial and eukaryotic proteins depend on zinc, non-heme iron or copper (1-3). Free Zinc is calculated as in femtomolar and copper in zeptomolar concentration in *Escherichia coli* cells (4,5). This predict that nearly all biometals are tightly bond in cells.
This investigation focused on the metal content of cells and the ratio of transition metals. ICP-MS was used to determine the biometal content of whole cells. We could show that the transition metal ratio in Gram-negative bacteria in exponential phase of growth does not depend on the growth medium used, from complex to minimal media. This ratio, however, changed in comparison to Gram-positive bacteria. A connection exists

between the predicted proteasome and the content of biometal co-factors. Only bacteria, which harbors manganese depend superoxide dismutase and members of NRAMP-family uptake system, contain a high amount of manganese (6). Additionally, the transition metal ratios change dramatically from bacteria to yeast and to phototrophic organism.

FTP059

Dissemination of dairy bacteriophages in whey powder samples

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Bacteriophages (phages) are a frequent cause for fermentation problems in dairies. When these bacterial viruses infect lactic acid bacteria, which are important lactic acid and flavour producers, serious delays or even complete failures of fermentation batches may occur resulting in significant financial losses. We have previously shown that phages of *Lactococcus lactis* starter strains may reveal remarkably high thermal stability [1]. Hence, these phages are not inactivated properly by heat treatment conditions used for pasteurization of raw milk and may propagate in the dairy resulting in high phage numbers in cheese whey (i.e., up to 10^9 plaque-forming units [pfu] per ml). Since large quantities of whey are processed into whey powder by spray-drying, we wanted to assess the dissemination of surviving dairy phages in whey powder samples.
Whey powder samples obtained from 11 whey powder producing plants were tested with a representative set of 59 starter strains encompassing mesophilic (*L. lactis*) and thermophilic (*Streptococcus thermophilus*) acid-producing and mesophilic flavour-producing bacterial isolates (*Leuconostoc pseudomesenteroides, Leuconostoc mesenteroides*). Notably, lytic lactococcal phages were present in samples from all 11 dairies, while *S. thermophilus* phages and *Ln. pseudomesenteroides* and *Ln. mesenteroides* phages were detected in samples obtained from 8 and 6 dairies, respectively. Maximal phage titers were $6x10^7$ (*L. lactis*), $1x10^6$ (*Ln. pseudomesenteroides*) and $1x10^4$ (*S. thermophilus*) pfu per gram of whey powder. Many of these phage populations revealed extended host ranges, illustrating the potential risk of re-cycling phage-contaminated whey components in dairy fermentation processes. Nearly all of the lactococcal phages isolated from whey powder belonged to the wide-spread 936 phage species known to include also the most heat-resistant phages.
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FTP060

Expression of subunit ND5 of the respiratory complex I (NADH:quinone oxidoreductase) from *Yarrowia lipolytica* in *Saccharomyces cerevisiae* leads to an increased salt sensitivity

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Complex I in the inner mitochondrial membrane is the first enzyme of the electron transport chain. Electrons from the oxidation of NADH are transferred to ubiquinone. This might induce conformational changes coupled to the transport of protons or other cations across the inner membrane. Our special interest is the ND5 subunit of the mammalian complex I, which is known to play a role in neurodegenerative diseases. The membrane bound ND5 subunit is considered to be involved in the proton pumping process and there is increasing evidence that ND5, also a homologue of bacterial H^+/Na^+ antiporters, is also able to translocate sodium ions (Gemperli, Schaffitzel et al. 2007). In addition human ND5 expressed in *S. cerevisiae* leads to an increased salt sensitivity (Steffen, Gemperli et al. 2010). In this study the optical density and the number of colony forming units (CFU) of *S. cerevisiae,* containing wild type and the F123L and E144G variants of ND5 from *Y. lipolytica* in membranes of the ER, were determined after incubation in a medium with or without added salt for 24, 48 and 72 hours. Cells containing wildtype ND5 grown without added salt exhibited the highest cell densities up to OD_{600} = 20, corresponding to 1 x 10^8 colony forming units (CFU). Growth of *S. cerevisiae* containing wildtype ND5 was heavily impaired when 800 mM NaCl or KCl was present in the medium. This inhibitory effect was diminished with *S. cerevisiae* producing ND5-E144G. We observed a 20-fold increase in CFU at 800 mM NaCl or KCl compared to cells producing wildtype ND5. The results are in accord with a presumed cation transport activity which affects ion homeostasis in *S. cerevisiae*. Support for this notion comes from Na^+ transport studies with ND5 in vesicles derived from endomembranes of *S. cerevisiae*. Wild type ND5 promoted Na^+ uptake (50 nmol after 90 sec) whereas vesicles containing the ND5-E144G variant exhibited diminished Na^+ transport activity (10 nmol after 90 sec). Thus, we conclude that the individual ND5 subunit of complex I from *Y. lipolytica* exhibits cation transport activity.

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FTP061

Structural characterization of lantibiotic immunity proteins

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Lantibiotics are peptide-derived antibiotics that inhibit the growth of Gram-positive bacteria mainly *via* interactions with lipid II and lipid II-dependent pore formation in the bacterial membrane. Due to their general mode of action the Gram-positive producer strains need to express immunity proteins (LanI proteins) for protection against their own lantibiotics. Subtilin producing *Bacillus subtilis* and nisin producing *Lactococcus lactis* strains possess four immunity genes which code for the lipoproteins SpaI and NisI and the ABC-transporter SpaFEG and NisFEG, respectively. Little is known about the immunity mechanism protecting the producer strain against its own lantibiotic on the molecular level.
The expression of SpaI or NisI alone is sufficient to confer subtilin immunity to *B. subtilis* or nisin immunity to *L. lactis*. Interestingly there is no cross-immunity between SpaI and NisI, despite the high sequence and structural similarity of subtilin and nisin but is in agreement with the limited sequence similarity between SpaI and NisI.
In order to elucidate this highly specific immunity mechanism we solved the structure of a 15 kDa biologically active fragment of SpaI by NMR which is the first structure of any LanI protein. NMR investigations of a full length construct of SpaI lacking the diacylglycerol anchor suggest that the 30 N-terminal amino acids are unfolded in the absence of a membrane. However, this N-terminal stretch interacts with liposomes in NMR titration experiments. When mutating this stretch *in vivo* the SpaI mediated immunity of *B. subtilis* against subtilin is not affected.[1]
The structure of the 25 kDa NisI protein in comparison to the 17 kDa SpaI will give insights to the highly specific immunity mechanism of LanI proteins. We were able to purify the full length NisI to high purity and stability for the structure determination by NMR. The ^{15}N-HSQC spectrum of NisI shows well dispersed peaks with some overlap in the center indicating a well folded protein with possible unfolded termini.
Our results are the first step on the way to understand the immunity mechanism of subtilin and nisin producing strains on a structural level at atomic resolution.

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FTP062

Application of encapsulated baker's yeast as attractant for soil living insect pests

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As several soil living important pest insects like western corn rootworm and wireworms use CO_2 for host (plant roots) location the use of attractants based on CO_2 is a promising approach for attract(-and-kill) strategies in pest control. Laboratory experiments have shown that different artificial CO_2

sources can attract larvae of the western corn rootworm and lure them away from the roots.
Hence there is a high interest in the systematic development of innovative plant protection products containing CO_2 sources as attractant with the purpose to achieve a long-time release of CO_2. Analogous to biological leaven products it is obvious to use microorganisms like baker's yeasts that release CO_2. It is of most importance to develop formulation methods to improve protection of the cells in soil, release of CO_2 and handling of the resulting product.
As CO_2 source, a commercial baker's yeast mixture or a pure *Saccharomyces cerevisiae* culture was encapsulated in moist Ca-alginate beads without and with additives and placed either in a closed chamber to determine CO_2 formation rates or in open boxes filled with soil to determine CO_2 amounts and gradients. We investigated the influence of different additives on the amount and the duration of CO_2 release with regard to the formation of CO_2 gradients in soil.
For the encapsulated baker's yeast mixture, experiments have shown a significant CO_2 release over 2 weeks which could be increased and prolonged by starch-containing nutrients by additional 2 weeks. The encapsulated yeast cells benefit from microbial contaminations in the yeast mixture or from soil microorganisms which provide amylases that catalyse the breakdown of starch into sugars. To guarantee a better reproducibility and the independence from microbial contaminations or soil microorganisms a pure *S. cerevisiae* culture was used in combination with starch containing nutrients and a synergistic entomopathogenic fungus as an amylase source. With the additives, the CO_2-release could be increased and prolonged over 5 weeks.
Further research will deal with the control of CO_2 release by systematic variation of encapsulation materials, methods and additives as well as glasshouse trials.

FTP063
Encapsulated plant extracts as antimicrobial agents against *Corynebacterium jeikeium*

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Because of the increasing resistance to drugs by some pathogenic microorganisms there is a renewed interest in the use of natural products like plant extracts as antimicrobial agents. It is of most importance to develop formulation methods that stabilize the active substances, control their release and provide an appropriate handling. For our encapsulation research we chose *Corynebacterium jeikeium* as an example for a multidrug-resistant microorganism. *C. jeikeium* is a "lipophilic" and multidrug-resistant bacterial species of the human skin flora. It is the most frequently recovered medically significant corynebacterial species at intensive care facilities and has been recognized with increasing frequency as a serious nosocomial pathogen.
In a first screening it was tested if lipophilic CO_2-extracts of plants can be encapsulated in hydrogel beads and if they show antibacterial potential against *C. jeikeium* in agar diffusion tests. The extracts were encapsulated in 2 % Ca-alginate beads (2.8 mm diameter) with or without emulsifiers. For *Origanum vulgare* (oregano) leaf extract, *Thymus vulgaris* (thyme) leaf extract and *Salvia triloba*/*Salvia officinalis* (sage) leaf extract agar diffusion tests showed considerable inhibitory effects for the pure extracts on filter discs and the corresponding encapsulated extracts. Generally, after three days the pure extracts showed greater inhibition zones than the corresponding beads. That is probably due to a slowed release of the active substances from the beads.
MICs (minimal inhibitorial concentrations) for plant extract emulsions were determined with a broth dilution method and were found to be 62,5 µg/mL for thyme, 50 µg/mL for oregano and 125 µg/mL for sage.
To investigate the release kinetic of the active ingredients encapsulated plant extracts were placed into aqueous media and incubated at 37 °C. The accumulation of active ingredients in the supernatant was tested by making use of the MIC test.
Further investigations will deal with the characterization of encapsulation materials, bead morphology and emulsifiers as well as their influence on the release kinetics.

FTP064
Structure of the GcpE (IspG)-MEcPP complex from *Thermus thermophilus*

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Biosynthesis of the two isoprenoids, isopentenyl diphosphate and dimethylallyl diphosphate, occurs through the mevalonate or the MEP pathway, the latter being exclusively utilized by most human pathogens, making the MEP pathway an attractive target for drug development. The penultimate reaction, the electron-driven conversion of 2-*C*-methyl-D-erythritol-2,4-cyclo-diphosphate (MEcPP) into *E*-1-hydroxy-2-methyl-but-2-enyl-4-diphosphate (HMBPP), is catalyzed by the iron-sulfur-cluster enzyme *E*-1-hydroxy-2-methyl-but-2-enyl-4-diphosphate synthase (GcpE). Each monomer (44 kDa) of GcpE is composed of a C-terminal TIM-barrel and the N-terminal [4Fe-4S] cluster binding α/β-domain, which are oriented in head-to-tails arrangement towards the domains of the second monomer. In its substrate-unbound state GcpE exhibits an open and flexible form [1]. Upon MEcPP binding a large scale 60° rotation of the [4Fe-4S] cluster binding domain onto the opening of the TIM-barrel funnel is induced, leading to a ligation of the apical iron of the [4Fe-4S] cluster with the C3 oxygen atom of MEcPP [2]. The substrate bound GcpE structure provides valuable insight into the catalytic mechanism.

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FTP065
Investigations on the molecular route of negamycin uptake

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Negamycin is a pseudopeptide which shows activity against gram-positive and gram-negative bacteria, including pathogens like *Staphylococcus aureus* and *Pseudomonas aeruginosa* (1). Negamycin is known to inhibit protein biosynthesis through a new and innovative dual mechanism. On the one hand it leads to miscoding and on the other it inhibits the termination process (2). Negamycin was shown to bind to the wall of the nascent exit tunnel of the 50S ribosomal subunit, but the mechanism of action is not understood on a molecular level, so far (3).
The antibacterial activity of negamycin strongly depends on the culture broth, suggesting an impact of the environment on negamycin uptake. Besides understanding the mode of action of a novel antibacterial agent, knowledge on its entry route into the bacterial cell is a prerequisite for further compound optimization.
In this context we investigate the active transport and the influence of salts and pH on the uptake of negamycin. In a previous study, the dipeptide permease Dpp was proposed as an uptake route for negamycin into *Escherichia coli* (4). Our results confirm this observation, but the remaining susceptibility of an *E. coli* Δ*dpp* strain indicates that there must be more than one entry route. We were able to show that negamycin is competing with peptides and the basic amino acid lysine for uptake. Furthermore, we observed a strong inhibitory effect of monovalent cations, and a strong improving effect of divalent cations on the activity of negamycin. An improvement of the negamycin MIC was also seen at a basic pH, suggesting that the zwitterionic antibiotic might be able to cross the cytoplasmic membrane by passive diffusion at this pH. Consequently, our findings show that negamycin enters the cell through several routes, which can be expected to reduce the risk of resistance development by transporter mutation.

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FTP066

ADEPs are active against Mycobacteria and target the ClpP1P2 complex of *M. tuberculosis*

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Acyldepsipeptides (ADEPs) are a novel class of antibiotics, acting through a unique mechanism by dysregulating the bacterial Clp protease. The proteolytic core of the protease (ClpP) is normally controlled by Clp-ATPases and adapter proteins. Binding of ADEPs to ClpP prevents its interaction with corresponding Clp-ATPases and leads to the inhibition of all natural functions of ClpP [1]. Additionally, ADEP binding leads to degradation of nascent polypeptides at the ribosome and non-native protein substrates in the absence of Clp-ATPases [2, 3].
Although ADEPs demonstrated promising antibacterial activity against several gram-positive pathogens, their therapeutic development was hampered by the fact that ClpP is not strictly essential in most bacterial genera, which leads to a frequent occurrence of ClpP mutations and ADEP resistance. In this study we are focusing on mycobacteria, because they encode two chromosomal copies of *clpP* and both are essential for growth [4]. As ADEPs demonstrated antibacterial activity against *Mycobacterium bovis* BCG, a widely employed model for *M. tuberculosis*, ADEPs are interesting lead structures for the development of new anti-TB drugs.
Recently, it was shown that ClpP1 and ClpP2 from *M. tuberculosis* interact to form a heterogeneous tetradecameric complex, where one heptamer is comprised by ClpP1 and the other heptamer by ClpP2. *In vitro* the presence of activating peptides such as benzyloxycarbonyl-leucyl-leucine (Z-LL) is required for the formation of the catalytically active ClpP1P2 complex [5]. We observed that ADEPs act on the ClpP1P2 complex of *M. tuberculosis in vitro* and that the antibiotics stimulate the degradation of structurally complex model peptides and unfolded proteins. Noteworthy, ADEPs exerted these effects only in the presence of Z-LL, which indicates different and additive mechanisms for the two activators. Thus, ADEPs are ideal tools to study the function of these unique ClpP proteins in mycobacteria, which represent promising new drug targets due to their essentiality in these organisms.

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FTP067

Studies on producer self-protection of *Streptomyces hawaiiensis* against antibiotic acyldepsipeptides (ADEP)

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Acyldepsipeptide antibiotics of the ADEP-class were discovered as secondary metabolites of the actinomycete *Streptomyces hawaiiensis* NRRL 15010 [1] and already showed promising antibacterial activity against Gram-positive pathogens *in vitro* and in infection models. They act at the unprecedented bacterial target ClpP, the proteolytic core of the caseinolytic protease. ADEP binding inhibits all physiological functions of ClpP by preventing the interaction with its associated Clp-ATPases. At the same time, it induces conformational changes in the ClpP core that leads to an opening of the entrance pore to the proteolytic chamber of the barrel-shaped ClpP tetradecamer and thus to an uncontrolled degradation capacity, finally causing bacterial cell death [2]. Thus, ADEPs represent powerful tools to explore the functions of Clp protease systems and their complex interplay with other cellular factors.
In this study, we investigate the mechanism of self-resistance of the ADEP producer strain *S. hawaiiensis*. Streptomycetes, which depend on a functional ClpP for viability [3], were shown to encode 5 different ClpP homologs. Previous studies using the ADEP-sensitive and non-producing *Streptomyces lividans* as a model system suggested, that the characteristics concerning ADEP-sensitivity vary between different ClpP homologs and that the expression of each homolog is tightly controlled by various regulators that are again partially dependent on regulatory proteolysis by the other ClpPs [3]. Like *S. lividans*, also the producer strain *S. hawaiiensis* contains various ClpPs. However, no data on their function, time course of expression and ADEP sensitivity are available, so far. Monitoring ADEP production during different culture phases and concomitant expression analysis of individual ClpPs and their regulators will help to obtain a better understanding of ClpP function in streptomycetes and to understand how *S. hawaiiensis* is protected against its own product.

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FTP068

MegxBar and PubMap: Storing and searching georeferenced scientific publications on microbes

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Scientific publications are the primary means of communicating scientific findings and data. The Internet is now the major publication platform, making it possible to access scientific publications directly or by searching literature databases like e.g. PubMed. However, extracting and structuring information of any given publication is a non-trivial and often tedious task. MegxBar and PubMap (Publication Map) are developed as part of the Marine Ecological Genomics web portal (megx.net) to aid in the manual curation and geographic visualization of scientific publications. MegxBar is a web browser extension for manual annotation of scientific publications. It gives users the ability to extract relevant information from a scientific article on the fly while reading the article in the browser. The main focus is on extracting the geographic origin of microbes and the contextual information according to the Minimum Information about any (x) Sequence (MIxS) standard introduced by the Genomic Standards Consortium. The extracted information is automatically structured and can be sent to the PubMap web service. The subsequent aim of PubMap is to provide an easy web-based system to geographically search publications and to offer geographic map visualizations.
Together, MegxBar and PubMap constitute the first literature database of georeferenced publications allowing researchers to find knowledge specific to their geographic area of interest.

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FTP069

Analysis of the biosynthesis of astins from *Aster tataricus* and cyclochlorotine from *Penicillium islandicum*

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Astins are cyclic pentapeptides isolated from roots of the plant *Aster tataricus*. The root extract shows potent anti-tumour activity in mouse assays. It is not known whether astins are produced by the plant itself or an associated entophyte (1). However, the amounts of astins that can be isolated from plants are very low and chemical synthesis is problematic. Therefore, the project ‚Multi enzyme systems involved in astin biosynthesis and their use in heterologous astin production (MESIAB)‘ aims at enhancing the production of astins using molecular genetic tools.
Cyclochlorotine, a secondary metabolite with high similarity to astins, has been isolated from the fungus *Penicillium islandicum*. Cyclochlorotine is a hepatotoxic compound causing necrosis, vacuolation of liver cells and development of blood lakes (2). Because of the high similarity of the peptides (3), similar enzymes should be involved in the biosynthetic pathways of astins and cyclochlorotine.
Both metabolites contain a dichlorinated pyrrole carboxylic acid derivative which is most likely derived from proline. The anticarcinogenic activity of astins relies on this chlorinated proline residue and on the cyclic structure of the peptide (4,5). It is assumed that chlorination occurs on the level of a

peptide carrier protein tethered pyrrol carboxylic acid moiety by a flavin-dependent halogenase. For genetic analysis the genome of *P. islandicum* was sequenced. A single gene for a potential halogenase was detected in the genome. This gene was cloned from cDNA and heterologously expressed in *E. coli* as a fusion protein with glutathione-*S*-transferase.

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FTP070
Investigation and characterization of putative interactions between penicillin-binding proteins and seds-proteins in sta-phylococci

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Staphylococci are Gram positive bacteria whose pathogenic species (e.g. *S. aureus*) are responsible for many diseases like pneumonia or endocarditis. The ability to acquire resistance to β-lactam antibiotics, which attack the bacterial cell wall or peptidoglycan, makes this strain particularly dangerous. In contrast, *S. carnosus* is a non-pathogenic food grade bacterium.
Peptidoglycan is the main component of the bacterial cell envelope, consisting of alternating N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc) subunits that are linked by a β-1, 4-glycosidic bond and cross-linked through the pentaglycine interpeptide bridges of the peptide subunits. The transglycosylation reaction fusing the GlcNAc and the MurNAc and the transpeptidation reaction between the peptide subunits are carried out byPenicillin-BindingProteins (PBPs).
Like all Eubacteria, staphylococci possess SEDS proteins (shape, elongation, division, and sporulation). A depletion of the SEDS protein RodA in *Bacillus subtilis* leads to spherical growth of the usually rod-shaped organisms. So far there is no definite function for the staphylococcal homologue of RodA, but the obtained *S. carnosus* deletion mutant shows growth defects.
To get a better insight into the process of the cell wall biosynthesis, PBP2 and RodA were screened against a genomic library of *S. carnosus* TM300 using the Bacterial-Two Hybrid (BTH) system. We found several interaction partners among the cell wall synthesising proteins that could also be confirmed in *S. aureus* N315. For example, we could show that RodA interacts with PBP1 to PBP4.
We are currently elucidating the part of RodA that might be responsible for interactions with other proteins. Therefore we are investigating different truncated RodA versions for their interaction activity with PBP1 to PBP4 in a BTH assay.
Localization studies in *S. carnosus* with an N-terminal fusion of GFP (greenfluorescentprotein) to RodA showed that this protein is exclusively located at the tip of the inward growing septum at the site of active peptidoglycan biosynthesis. First RodA accumulates at one side of the cell, later it is also found directly opposite of it. Then RodA moves from both directions to the center of the cell. This indicates a pivotal role for RodA during cell wall biosynthesis of staphylococci.

FTP071
Optimization of the precursor supply for the improved production of tacrolimus in *S. tsukubaensis*

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The enhancement of the productivity of *S. tsukubaensis* was achieved by genetic engineering of the wild-type producer. For the optimization of the precursor supply in order to achieve an improved tacrolimus production genes for the L-pipecolic acid biosynthesis, *fkbL* (encoding lysine cyclodeaminase) and *fkbP* (encoding pipecolic-activating non-ribosomal peptide synthase) from *S. tsukubaensis* have been over-expressed. Pipecolic acid is a constituent of a few secondary metabolites like pristinamycin, friulimycin and tacrolimus. The genes responsible for the conversion from lysine to pipecolic acid from *S. pristinaespiralis* and *A. friuliensis* have also been cloned. These genes (*pip*) are located in the pristinamycin and friulimycin biosynthetic gene clusters respectively and are under control of pathway-specific regulators. To optimize the supply of the building block pipecolic acid and thus increase the yield of the tacrolimus production the *pip* genes from *S. pristinaespiralis* and *A. friuliensis* were heterologously expressed in the tacrolimus producer *S. tsukubaensis*. For the heterologous expression of the genes coding for the lysine cyclodeaminases from *S. pristinaespiralis* (*pipA*) and *A. friuliensis* (*pip*) over-expression plasmids using the pGM1190 vector have been constructed and introduced into *S. tsukubaensis* via conjugation. Preliminary results showed that the over-expression of the *pip* genes positively influenced the tacrolimus production. The supplementation of the production medium with lysine (precursor of the pipecolic acid) also strongly enhanced the production of tacrolimus in *S. tsukubaensis*. Finally, the flux through the lysine biosynthesis was optimized by directly modifying the key enzymes aspartate kinase (LysC) and dihydrodipicolinate synthase (DapA).

FTP072
Uptake and dissipation of pah in legumes-microbe-soil system

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Polycyclic aromatic hydrocarbons (PAHs) uptake and dissipation in the root zone of legumes (Arachis hypogea, Cajanus cajan, and Lablab purpureus) were investigated. Plants were grown in soil contaminated with 1000, 10,000 and 50,000 mg/kg of PAHs and uncontaminated control soil. PAH content was determined in plant tissue and soil using high performance liquid chromatography (HPLC) with UV-VIS detector. PAH degrading bacteria were enumerated using plate dilution technique. Variability was observed in the uptake of the PAHs by the legumes. Naphthalene was neither detected in the plant tissues nor the soil. Anthracene was more accumulated in plant tissue grown in uncontaminated soil than phenanthrene. Concentration of anthracene detected in the shoot ranged between 1.27 mg/g for L. purpureus and 35.20 mg/g for A. hypogea. Phenanthrene was predominant in contaminated soil than anthracene with highest concentration of 49.55 mg/g detected in rhizosphere soil of L. purpureus. In C. cajan none of the PAHs was detected in plant tissue and soil with the exception of soil (1000mg/kg) which 43.65 mg/g of anthracene was detected. Greater rhizosphere effect was observed in the contaminated soil with counts of PAH degrading bacteria ranging from 2.6 ±0.16 x 107 to 9.0 ± 2.1x 107 cfu/g. Bacterial isolates identified belonged to Bacillus, Pseudomonas and Micrococcus species. The results indicate that both atmospheric and soil inputs contribute to PAHs in plants, suggesting that phytoaccumulation, phytostabilization and rhizodegradation are the main pathways of PAH remediation by these legumes.

FTP073
Novel epsilonproteobacterial symbionts in gill tissues of deep-sea hydrothermal vent mussels

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Mussels of the genus *Bathymodiolus* colonize deep-sea hydrothermal vents and cold seeps worldwide. They are well known for their beneficial associations with gammaproteobacterial endosymbionts, which provide them with nutrition. The mussels can host either sulfur oxidizers that use sulfide as an energy source for carbon dioxide fixation, or methane oxidizers that use methane as an energy and a carbon source, or both in a dual symbiosis. The gammaproteobacterial symbionts are hosted in gill tissues, where the mussel host provides them with their sources of nutrition by pumping sulfide- and methane-rich fluids across the gills. We recently discovered a novel epsilonproteobacterial sequence in 16S rRNA clone libraries from *Bathymodiolus* gill tissues. The *Bathymodiolus* epsilonproteobacterial sequences are related to the sulfur oxidizer *Sulfurovum lithotrophicum*, and cluster together with the ectosymbionts of other hydrothermal vent animals such as shrimp and crabs. We have now found closely related epsilonproteobacterial sequences in clone libraries from 4 *Bathymodiolus* species from 4 different vent and seep sites covering an enormous geographic range from the Golf of Mexico to the Sagami Bay, indicating that this association is widespread. To further investigate this novel symbiosis, we designed specific probes for fluorescence in situ hybridization (FISH). FISH of *Bathymodiolus childressi* from cold seeps in the Gulf of Mexico showed that, like their close relatives involved in other symbiosis, the *Bathymodiolus* epsilonproteobacterial are ectosymbionts, found attached to the gills. Intriguingly, the epsilonproteobacterial ectosymbionts preferentially colonize the external ciliated edges of mussel gill filaments, which are the first parts of the gills to be exposed to sulfide-

rich fluids. In contrast this region is never colonized by the gammaproteobacterial endosymbionts. These recent insights suggest that the diversity of the symbiotic community of *Bathymodiolus* mussels might have been underestimated. Although we do not know the role of the epsilonproteobacterial epibionts in the *Bathymodiolus* symbiosis, the presence of closely related ectosymbionts on diverse host species from vents and seeps around the world suggests that the association is specific and might therefore be beneficial.

FTP074

Novel fluorescent reporter proteins for the *in vivo* analysis of biological processes

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Fluorescent reporter proteins (FPs) like the green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* enable the non-invasive quantitative real-time analysis of complex cellular processes *in vivo*. However, a major drawback of GFP and its variants is their strict limitation to aerobic biological systems. This is primarily due to the fact that the autocatalytic synthesis of their fluorophores strictly depends on molecular oxygen. Therefore, we recently developed a new class of fluorescent proteins which can be used under aerobic as well as oxygen-limited and anaerobic conditions [(1,2,3)]. These FPs carry flavin mononucleotide (FMN) as the fluorophore and are thus termed FMN-binding fluorescent proteins (FbFPs). Beside cell labeling, analysis of gene regulation and protein localization, FbFPs can also be used to generate novel molecular biosensors with unique properties allowing the *in vivo* measurement of essential environmental parameters with high spatio-temporal resolution[4].

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FTP075

Phylogeny and geography in the ciliate *Kentrophoros* and its thiotrophic symbionts

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Chemolithoautotrophic sulfur-oxidizing bacteria (thiotrophs) are often found in symbiosis with eukaryotic hosts. The best-known host organisms are animals (Metazoa) but such symbionts are also found with ciliates (Ciliophora).
Two ciliate taxa are known to have thiotrophic ectosymbionts: the sessile colonial *Zoothamnium*, and the free-living solitary *Kentrophoros*. While *Zoothamnium* prefers hard substrates, *Kentrophoros* inhabits the interstitial porewater of shallow-water sediments. The genus *Kentrophoros* comprises about 15 species, which have been described from all major oceans. The cells have a flattened vermiform shape, like many other interstitial ciliates, and are dorsally covered with ectosymbionts. Previous studies have shown that the ectosymbionts can oxidize sulfide and fix carbon, but the pylogenetic affiliation of the bacteria is unknown.
For this study, we collected *Kentrophoros* from the islands of Sylt (North Sea) and Elba (Mediterranean). We use molecular markers (18S and 16S rRNA genes) to address the following questions: (1) Are the ectosymbionts phylogenetically nested among known thiotrophic symbiont clades or do they represent a separate lineage? (2) Is there a pattern of host-symbiont co-diversification? (3) Is there geographical differentiation in *Kentrophoros* and its symbionts?
As a widely-distributed microbial eukaryote with microbial symbionts, *Kentrophoros* is a promising system to test hypotheses about microbial biogeography. As a ciliate, it presents the opportunity to see whether lessons learned from animal-bacterial symbioses also hold true for other eukaryotic-bacterial symbioses.

FTP076

Molecular transport across the hetero-oligomeric cell wall porins derived from Gram positive bacterium *Nocardia farcinica*

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Selective and controlled transport of substances to and from the cells are regulated by various protein channels present in the cell membrane. For instance, membrane of gram-negative bacteria contains different channels of nanometer dimensions that are either specific to a certain molecule, eg. Maltoporin to maltose, or allow a wide range of molecules to pass through them, eg. OmpF. In contrast, gram-positive bacteria do not have large pore forming proteins in their outer membrane. However, it has been studied that the class of bacteria such as Mycobateria, Corynebacteria, Nocardia belonging to the order of *actinomycetales* from Gram-positive bacteria have channel forming proteins in their outer membrane mycolic acid layer. In this work we characterize the transport of macromolecules across the cell wall porins of *Nocardia farcinica,* known to be a dangerous pathogen causing Nocardiosis. An adequate method to study properties of these channels is electrophysiology and in particular analyzing the ion current fluctuation in the presence of permeating solutes provides information on possible interactions with the channel surface.
The translocation of small solutes, such as sugars, peptides and antibiotics, through the channel has been studied by ion current fluctuation analysis at single molecular level.

FTP077

Efficacy of plant growth promoting rhizobacteria having diverse growth promoting traits for alleviating salinity induced impact on cucumber growth

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Phosphate solubilization, ACC-deaminase activity, siderophores and indole acetic acid production are well known traits of plant growth promoting rhizobacteria (PGPR). There is little information about comparative effectiveness of these traits for promoting plant growth under stress conditions. The aim of this study was to evaluate the potential of PGPR regarding these traits under normal and stress conditions as well as to find out the comparative performance of PGPR for alleviating salinity induce negative impact on cucumber growth. Three pre isolated PGPR strains (*Variovorax paradoxus*, *Pseudomonas fluorescens* and *Bacillus megaterium*) were characterized for salinity tolerance, ACC-deaminase activity, phosphate solubilization, siderophores and indole acetic acid production under normal and saline conditions. The strains showed great variability regarding salinity tolerance and activity of growth promoting traits also varied under normal and stress environment. *B. megaterium* showed significant tolerance against salinity (5% NaCl) compare to *P. fluorescens* however, it loosed its ability to maintain its original level of ACC-deaminase activity, siderophores and indole acetic acid production under stress conditions. *V. paradoxus* was unable to grow at high salinity and also showed less potential regarding other traits under stress. The impact of these strains on cucumber growth under three salinity levels i.e normal, 7 and 10 dS/m also showed variable results. Although in preliminary studies conducted in the laboratory, *B. megaterium* showed more tolerance to increasing level of salinity however, *P. fluorescens* showed maximum root colonization and proved more effective for alleviating the negative impact of salinity on cucumber. Cucumber growth and physiological parameters were significantly improved by *P. fluorescens* inoculation at low and high salinity. The results shows that in addition to salinity tolerance ability of a PGPR strain, the activity of growth promoting traits under stress are also very important aspect. Such information could be very useful for selection of an effective PGPR to get maximum benefits from this naturally occurring population for promoting plant growth under stress conditions.

FTP078

Activation of acetone by strictly anaerobic bacteria

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Acetone is activated by aerobic and nitrate-reducing bacteria to acetoacetate as the first reaction product. The overall reaction consumes at least 2 ATP equivalents. This highly energy-consuming reaction is not likely to occur in sulfate-reducing bacteria due to their energy limitation. In this work the activation of acetone by sulfate-reducing bacteria is being studied. Several lines of indirect evidence indicate that acetoacetate is not an intermediate in this degradation pathway. Instead, we found hints for a "carbonylation reaction" as the initial step in the activation of acetone. Among others, we found indications of the addition of carbon monoxide to the acetone molecule to form acetoacetaldehyde as intermediate. This concept is supported by experimental results obtained with cell suspension experiments combined with the detection of reaction products by electrospray ionization-mass spectrometry. We observed that the expected product of the carbonylation is highly unstable. The polymerization of acetoacetaldehyde has been reported before to lead to triacetylbenzene, a compound that we were able to identify by mass spectrometry.
The genome of the sulfate-reducing bacterium *Desulfoccus biacutus* has been sequenced and automatically annotated. According to the pattern of acetone-induced proteins and enzyme tests a thiamine pyrophosphate requiring enzyme is involved in the acetone activation. However, the activation mechanism is still under investigation.

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FTP079

Characterization of the JEN-family of carboxylate transporters in *Yarrowia lipolytica*

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The yeast *Yarrowia lipolytica* secretes high amounts of various organic acids, like citric, isocitric, pyruvic and a-ketoglutaric acids, triggered by growth limitation and excess of carbon source. This is leading to an increased interest in this non-conventional yeast for biotechnological applications. In this regard the metabolism and transport of these carboxylic acids is a main point of interest. Undissociated forms can cross the membrane by simple diffusion. In contrast the uptake of dissociated forms requires a mediating mechanism. In cases of *Saccharomyces cerevisiae* (*JEN1*) and *Kluyveromyces lactis* (*KlJEN1*, *KlJEN2*) a few of such mediated transport systems were already reported. Sequence alignments in *Yarrowia lipolytica* resulted in detection of a family of six putative carboxylate transporters (*YlJEN1-YlJEN6*) [1]. Here we report a first physiological and molecular characterization of these transporters, including examinations to potential carbon catabolite repression and effects on the production of organic acids as well as growth on different carboxylic acids in deletion strains.

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FTP080

Bacteriochlorophyll Biosynthesis: Chlorophyllide *a* Oxidoreductase from *Roseobacter denitrificans*

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Chlorophyllide *a* oxidoreductase (COR) catalyzes the stereospecific reduction of the C-7/C-8 double bond of ring B during bacteriochlorophyll biosynthesis. Chlorophyllide *a* (Chlide) is reduced in an ATP-dependent process into 3-vinyl bacteriochlorophyllide *a* (Bchlide) [1]. The enzyme is composed of three protein subunits termed BchX, BchY and BchZ. All subunits show significant homology to the corresponding subunits of nitrogenase (NifH, NifD and NifK) and also to dark-operative protochlorophyllide oxidoreductase (DPOR; BchL, BchN and BchB) [2].
All COR subunits were heterologously overproduced in *E. coli*. Due to the tight interaction of BchY with BchZ both subunits were co-purified by using His_6-tagged BchY. Subunit BchX was produced separately. Gel permeation chromatography analysis indicated a heterotetrameric architecture of $(BchY/BchZ)_2$ and a homodimeric quaternary structure for subunit $BchX_2$ [2]. The UV/Vis absorption spectra of $(BchY/BchZ)_2$ and also of $BchX_2$ showed an absorption maximum at 425nm indicative for the presence of [4Fe-4S] clusters [2]. EPR studies indicated a specific signal for a [4Fe-4S] cluster located on $BchX_2$.
Mutagenesis experiments in combination with *in vitro* COR activity assays revealed an inter-subunit [4Fe-4S] cluster coordinated by three cysteinyl ligands located on BchY and an additional cysteinyl ligand located on subunit BchZ.
For the analysis of COR substrate recognition seven artificial substrates were tested *in vitro* [3, 4]. The experiments using Zn-coordinating molecules with differing ring substituents indicated that the COR system tolerates minor modifications of substituents on rings A and E, whereas no modification of ring D was accepted.

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FTP081

How to shift and exploit the unfavourable equilibrium of ADPC formation

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The recently discovered compatible solute ADPC (5-amino-3,4-dihydro-2H-pyrrole-2-carboxylate) was shown to be the product of a side reaction of ectoine synthase (EC 4.2.1.108), normally responsible for ectoine biosynthesis in halophilic microorganisms [1]. The cyclic condensation of glutamine, resulting in ADPC, was shown to be reversible with the equilibrium largely on the side of the educt glutamine. This proved to be quite a challenge in terms of ADPC production as only low intracellular ADPC levels can be reached.
Our ongoing studies focus on the optimization of ADPC production, using two different strategies which both depend on removing the product from the educt.
Strategy 1 (increased membrane permeability of the producer strain):
As has been shown before, ectoine production in *H. elongata* can be enhanced by deleting the ectoine transport system TeaABC, resulting in ectoine leakage into the medium and subsequent overproduction [2]. We were able to demonstrate that ADPC formation in such "leaky" strains deprived the equilibrium reaction of the product and enhanced the accumulation of ADPC in the medium.
Strategy 2 (uptake of the product by accumulating strain):
As a challenging alternative we also investigated the possibility to combine *in vitro* production of ADPC from glutamine by the purified enzyme with subsequent uptake and accumulation of the product into salt-stressed bacterial cells, which are unable to metabolize ADPC. The second option has the advantage that the desired product could be recovered from biomass via "bacterial milking" [3].

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FTP082

Interactions of cationic peptides with a protein pore at a single-molecule level

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Most antimicrobial peptides are cationic peptides (CAPs) composed of 12-50 amino acids with a net positive charge of about +4 to +6. CAPs have effect against both gram +ve and -ve bacteria. Here, the properties of interactions of short cationic peptides with protein pores reconstituted into artificial lipid bilayer has been studied using electrophysiology. Noise analysis of ion currents through a protein channel, OmpF in the presence of peptides reveal binding constants and transport parameters at a single molecule level. We have investigated the concentration and voltage dependence of the membrane transport of the cationic peptides through single protein channel. Further we conjugated the short cationic peptide with PEG, a hydrophilic and highly flexible polymer. In the presence of the small cationic peptides conjugated with differently sized polymers, the ionic conductance of the channel demonstrates well-defined transitions between open and closed state demonstrating either binding or translocation of peptide. Our analysis of the data provides a quantitative description of the peptide partition through a biological channel at single molecular level.

FTP083

Evaluation of MALDI-TOF MS for identification of microorganisms of veterinary origin

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Objectives: Identification of microorganisms of veterinary origin is usually done by time consuming cultural and biochemical tests or by semi automated methods. These tests need a lot of consumables and laboratory staff has to be highly qualified. MALDI-TOF MS is a fast, reliable and easy to handle alternative method for identification of microorganisms and widely used in clinical diagnostics. The MALDI Biotyper system (Bruker Daltonik) was evaluated for identification of 44 different strains obtained from veterinary routine diagnostic.
Methods: Field isolates were previously identified with morphological and biochemical tests /API test system or with species-specific PCR. Microorganisms were cultivated and colonies were suspended in 75% ethanol. Formic acid/acetonitrile extraction method was used. Mass spectra were collected by a microflex. MALDI Biotyper 3.1 software and database version 3.3.1.0 with 4613 references was used for identification.
Results: Bacteria analysed in this study included strains of genera *Actinobacillus*, *Avibacterium*, *Bordetella*, *Erysipelothrix*, *Gallibacterium*, *Haemophilus*, *Ornithobacterium*, *Riemerella*, *Staphylococcus*, *Streptococcus*, and *Trueperella*. A total of 43 strains (97.73%) were identified to species level. Biochemical/PCR and MALDI Biotyper results agreed at species level for 42 isolates. Only an *Avibacterium gallinarum* isolate was assigned to *Avibacterium endocarditidis* with a log(score) value of 2.047 using MALDI Biotyper. A 16S rRNA sequencing approach of this sample failed to get a sequence. A presumed *Riemerella anatipestifer* isolate was clearly distinguished from all other eight *R. anatipestifer* strains also analysed in this study. In line to this, 16S rRNA gene sequence of this isolate was similar to *Riemerella columbipharyngis* (97.3% similarity) and *Chryseobacterium flavum* (97.3% similarity).
Conclusions: The results displayed MALDI-TOF MS as a fast and reliable automated method for identification of these species of veterinary origin.

FTP084

Functional dissection of the tetracysteine motif of the natural competence ATPase PilF in *Thermus thermophilus* HB27

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Thermus thermophilus is known for its high natural competence and the ability to move on moist surfaces by twitching motility. The latter is mediated by type 4 pili (T4P). Several components of the DNA translocator share similarity to T4P proteins, such as pilins, secretins and a motor ATPase (1,2). This together with the finding that several DNA translocator proteins exhibit a dual function in both, T4P and DNA uptake suggests that both systems are linked.
Recently, we have shown that the AAA-ATPase, PilF, which is essential for natural transformation of *T. thermophilus*, is a unique, zinc containing transport ATPase forming a homooligomer of six subunits (3). Each PilF monomer was found to comprise three general secretory pathway domains (GSPII) and a tetracysteine motif. The tetracysteine motif was found to mediate zinc binding (3).
To analyze the functional and structural role of the cysteine residues we generated *pilF* mutants and performed mutant studies. These studies revealed that none of the cysteine residues is essential for natural transformation and ATPase activity. In contrast several cysteine residues were found to be essential for piliation and therefore for pilus mediated functions such as twitching motility and adherence. Taken together these findings suggest that the T4P structures are not essential for natural transformation.
To address the structural role of the tetracysteine motif we analyzed the effect of the mutations on heat stability of the PilF hexamer. These studies revealed, that the mutated PilF proteins were significally reduced in heat stability of PilF complexes suggesting a structural role of the tetracysteine motif essential for complex stability.

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FTP085

Analysis of LanI mediated lantibiotic immunity in *Bacillus subtilis*

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A widespread mechanism of bacteria to compete against phylogenetically closely related organisms is the production of so called bacteriocines. An important class of these bacteriocines are the lantibiotics [1]. *Bacillus subtilis* ATCC 6633 produces the lantibiotic subtilin, which acts on Gram-positive microorganisms by interfering with the lipid II cycle [2]. Self protection of the producer cells is mediated by the lipoprotein SpaI and the ABC-transporter SpaFEG [3]. The structure of a 15 kDa biologically active fragment of SpaI was solved by NMR and different SpaI mutations were generated to elucidate the mechanism of SpaI-mediated immunity [4]. The results showed that the N-terminus of SpaI is able to interact with liposomes *in vitro*. In accordance with this we found that the charge of the N-terminus is important for the maturation of the protein.

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FTP086

Quantification of IncP-1 plasmid dynamics in on-farm biopurification systems for pesticide waste

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Broad-host-range plasmids of the incompatibility group IncP-1 are assumed to play an important role in bacterial adaptability to environmental challenges or opportunities. Besides a conserved backbone carrying genes for plasmid persistence and conjugative transfer, diverse accessory genes are described for IncP-1 plasmids encoding resistance to antibiotics or heavy metals as well as degradative pathways. However, the environmental distribution of IncP-1 plasmids and the factors promoting their frequency in bacterial communities are not well explored. Recently, a TaqMan® real-time quantitative PCR assay was developed for the IncP-1ε subgroup based on the *trfA* gene, which codes for the replication initiation protein [1]. However, the quantification of all known IncP-1 subgroups (α, β, γ, δ, ε and

ζ) based on the *trfA* gene is problematic due to its high evolutionary rate and thus the lack of conserved targets for primers and a TaqMan® probe. In this study, a real time PCR 5' nuclease assay based on the detection of the conserved regulatory and partitioning gene *korB* was developed to quantify IncP-1 plasmids of all known subgroups. The broader specificity was confirmed by the analysis of environmental samples from a pesticide-degrading biofilter. Samples were taken over a season with continuing applications of pesticide polluted water and the *korB* assay detected 2 to 4 times more IncP-1 plasmids than the *trfAε* assay. The relative abundance of IncP-1 plasmids in the bacterial biofilter community increased from March till September reaching values of up to 0.2%, which might indicate an important contribution of degradative genes carried on IncP-1 plasmids to pesticide degradation.

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FTP087

Arabitol metabolism in *Corynebacterium glutamicum* and its regulation by AtlR

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Corynebacterium glutamicum is a Gram-positive soil bacterium, which gains increasing significance for biotechnological applications because of its capability to produce amino acids. Previous studies on regulation of ethanol metabolism revealed the AtlR protein as a Deo-R type transcriptional regulator affecting expression of the ethanol dehydrogenase gene *adhA*. Subsequent DNA-microarray experiments with *C. glutamicum* wildtype (WT) and *C. glutamicum* Δ*atlR* revealed that expression of four adjacent genes putatively encoding a ribitol transporter (RbtT), a mannitol 2-dehydrogenase (MtlD), a xylulose kinase (XylB) and a phosphohistidine phosphatase (*rbtT*, *mtlD, xylB*, and *sixA*, respectively) was positively affected by deletion of *atlR*. Transcriptional analysis indicated that *atlR* and the four genes are organized as *atlR-xylB* and *rbtT-mtlD-sixA* operons. Growth experiments revealed that (i) *C. glutamicum* WT efficiently grows on D-arabitol, but not on other sugar alcohols, (ii) RbtT is involved in D-arabitol transport, (iii) MtlD and XylB are essential for D-arabitol metabolism, and (iv) SixA is not required for growth on D-arabitol. Furthermore, *C. glutamicum* WT showed high mRNA-levels of *atlR, rbtT*, *mtlD, xylB*, and *sixA* during growth on D-arabitol. In accordance to the latter result, we show that MtlD confers D-arabitol dehydrogenase activities, and that these as well as XylB activities are generally high when the cells grow in the presence of D-arabitol and very low when they grow in its absence. In contrast, *C. glutamicum* Δ*atlR* displays generally very high MtlD and XylB activities independent on the carbon source the cells had grown on. Significantly increased mRNA-levels of *rbtT*, *mtlD, xylB*, and *sixA* in glucose grown *C. glutamicum* Δ*atlR* cells corroborate the finding that the transcriptional regulator AtlR is involved in regulation of arabitol metabolism in *C. glutamicum* by repression of the *atlR-xylB* and the *rbtT-mtlD-sixA* operons in the absence of D-arabitol.

FTP088

Production of new bioactive compounds by plants and bacteria using new and improved halogenases

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Natural products with wide-ranging biological activities are produced by many organisms. The biological activity and/or the bioavailability of these compounds often depend on the pattern of substituents in these molecules. One modification that normally has a large activity-increasing effect is halogenation (chlorination and bromination) by flavin-dependent halogenases.
Flavin-dependent tryptophan halogenases, apart from their high substrate specificity, show an extremely high regioselectivity: Tryptophan halogenases with regioselectivities for the 5-, 6-, and 7-position, respectively, have recently been isolated and characterized in some detail. A tryptophan halogenase with another regioselectivity, namely for the 4-position, is supposed to be present in higher plants producing the halogenated plant hormone 4-chloroindole-3-acetic acid.
In our new project, we will identify and clone the gene of a tryptophan 4-halogenase from *Pisum sativum*, heterologously express it and biochemically characterize the corresponding protein. On the basis of the 3D-structure of known tryptophan halogenases, we will try to create halogenase variants with an expanded substrate specificity. The native tryptophan halogenase genes as well as the created mutant genes will be introduced into different secondary metabolite producing bacteria and plants and into plants producing high levels of indole-3-acetic acid.
The new halogenated metabolites obtained by pathway modification will be screened for their biological activities and the *in vivo* effects of new chlorinated indole-3-acetic acids as plant growth promoting compounds will be studied in model and crop plants.

FTP089

Insight into the mechanism of Super-oxide production by the Na^+ - NQR (NADH:Quinone Oxidoreductase) from *Vibrio cholerae*

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The marine bacterium V. cholerae is mainly prevalent in the tropical sea or in brackish waters of river outfalls. The high content of sodium in its natural habitats leads to a high sodium gradient between the bacterial cell and its environment. V. cholerae, as well as other marine bacteria, is able to use this sodium gradient for the energetization of different metabolic processes, e.g. the rotation of its flagellum or the transport of various substrates[1].
To prevent the accumulation of sodium ions in the cell, the bacterium constantly has to remove the intracellular sodium ions to maintain the gradient and thus can benefit from the sodium motive force.
In V. cholerae, this active transport is achieved by means of the Na+ - NQR. The Na+ - NQR is a membrane-bound enzyme complex composed of six subunits (NqrABCDEF) which contains four flavins, one 2Fe-2S cluster and ubiquinone-8 as cofactors. Being part of the respiratory chain, the Na+ - NQR complex couples the oxidation of NADH with Ubiquione-8, to the transport of sodium ions across the inner membrane [2].
A side effect of this reaction is the formation of reactive oxygen species (ROS), which lead to severe cell damage and constrained phenotypes. Recently the Na+ - NQR could be identified as the main source of superoxide in V. cholerae, although the exact mechanism could not be clarified [3].
To elucidate the exact mechanism of the formation of ROS within the NQR complex, and in order to localize the site of their formation, we used several in vitro and in vivo approaches with truncated versions of the Na+ - NQR complex which lack individual subunits. In this way we gained insight in this mechanism and were able to narrow down the site of ROS formation.

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FTP090

Effect of fermentation residues on the soil microbial communities and soil organic matter carbon after application on arable soils

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Atmospheric CO_2 level has increased dramatically during the last two centuries. An important portion of anthropogenic CO_2 is generated by the mineralization of soil organic matter (SOM) due to land use changes. For example, the increased cultivation of bioenergy crops reduces the carbon input from plant residues on agricultural areas. A suitable organic soil amendment to avoid soil degradation on these areas is application of biogas residues (BGR) which are side products of biogas production. Application of BGR as soil additive influences the soil microorganisms as well as the composition and the turnover of soil organic matter.
This effect will be studied by incubating ^{13}C-labeled BGR in an arable soil from the Static Fertilization Experiment in Bad Lauchstädt, Germany. For producing these labeled residues, $KH^{13}CO_3$ is added to biogas reactors in order to label the active microbial biomass. The labeled fermentation residues will then be incubated in the soil for 1 year in the laboratory.

During incubation, the dynamics of the microbial community during soil incubation will be determined using molecular tools. 16S rDNA clone libraries will be constructed and denaturing gradient gel electrophoresis will be used to monitor the communities. Quantification of 16S rRNA and its coding genes with group-specific primers are also planned. Additionally, the fate of the labeled carbon will be monitored and its effects on SOM turnover and soil characteristics will be investigated. The methods will include isotope mass balances and analysis of the concentration and isotopic composition of phospholipid fatty acids and amino acids. This will allow to trace the fate of the BGR-derived C in soil as well as to quantify the effect of the BGR on the transformation of the natural SOM (e.g. priming effects). For improving potentially negative effects, various additives (charred biomaterials, clays, chopped bark) will be tested to improve the carbon storage.
In conclusion, this study will investigate the impact of BGR used as a soil additive on the soil microbial community and amount of SOM thus contribute to recommendations about the use of BGR as fertilizers or soil additives in agriculture. Furthermore, the data will be a potential input to a landscape generator model which will predict soil quality development in Central Germany.

FTP091

Heterologous expression of a *Photorhabdus luminescens* syrbactin-like gene cluster results in production of the potent proteasome inhibitor glidobactin A

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Syrbactins are a group of cyclic peptide derivatives which have been shown to form irreversible covalent bonds with catalytic sites of the eukaryotic proteasome, thus inhibiting its activity (Groll et al., 2008). The family consists of two members characterized to date, namely syringolin A and glidobactin A, which are produced by several strains of *Pseudomonas syringae* and strain K481-B101 from the order *Burkholderiales,* respectively. Syrbactins are the products of mixed non-ribosomal peptide/polyketide synthetases arranged in gene clusters with a characteristic architecture (Amrein et al., 2004; Schellenberg et al., 2007). Similar, but not identical gene clusters have been identified in several other unrelated bacterial genomes, including that of *Photorhabdus luminescens* subsp. *laumondii* TT01, which is therefore hypothesized to be able to produce a syrbactin-type proteasome inhibitor. Due to fact that it was not possible to identify cultivation conditions for *Ph. luminescens* inducing expression of the putative syrbactin synthetase-encoding gene cluster, the respective genomic region has been cloned into a cosmid vector and heterologously expressed in *Pseudomonas putida*. Analysis of culture supernatants of transformed *Ps. putida* by liquid chromatography electrospray ionisation tandem mass spectrometry (UPLC-ESI-MS/MS) revealed the presence of glidobactin A, indicating that the syrbactin-like gene cluster of *Ph. luminescens* encodes a glidobactin A synthetase and that this organism has the capacity to produce this proteasome inhibitor.
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FTP092

Accumulation of Cry1Ab/Ac proteins from subspecies of *Bacillus thuringiensis* in medium and different paddy soils

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The gram-positive bacterium *Bacillus thuringiensis* (*Bt*) can produce parasporal proteinaceous crystals during sporulation which consists of one or more insecticidal proteins (Cry and Cyt proteins). Four subspecies of *Bt* were used to study cell growth in a sporulation medium, two of which, *Bt* ssp. *thuringiensis* and ssp. *kurstaki*, were confirmed to produce Cry1Ab/Ac proteins. The strains were grown until stationary phase.
After *Bt* strains were inoculated into the liquid medium, the exponential and slow-down phase of the stains took 36 h. During this period, Cry1Ab/Ac proteins (Bt toxin) from two *Bt* strains started accumulating in the medium. Moreover Bt toxin was increasing rapidly during the stationary phase, immediately after the exponential and slow-down phase. Rapid increase in cells of *Bt* strains and Cry1Ab/Ac proteins didn't start at the same time.
In a further experiment the *Bt* ssp. *thuringiensis* and ssp. *kurstaki* were used to study Cry1Ab/Ac accumulation in the sterilized/non-sterilized soil (four paddy soils of different texture: TL, TX, MC and LC), respectively. The strains were collected by centrifugation, diluted in H_2O_{MQ} and then added to the soils. In the soil inoculated with the strain *Bt ssp. thuringiensis*, concentrations of Bt toxin started to decrease 19 days after the inoculation. In the soil inoculated with *Bt* ssp. *kurstaki*, Bt toxin accumulated continuously after the inoculation. Concentrations of Cry1Ab/Ac protein were higher in the TL and TX soil than that in LC and MC soil. For the sterilized four soils, Bt toxins accumulated more than that in the non-sterilized four soils.
After centrifugation, Cry1Ab/Ac protein from *Bt* ssp. *kurstaki* in the supernatant was collected. An aliquot of Cry1Ab/Ac protein solution (0.92 μg/ml) in medium was mixed with LC, MC, TX, or TL soil and rotated for 24 h at room temperature. The Bt toxin could be adsorbed into the soil. The amount of Bt toxin adsorbed was different, with the increasing order: LC<MC<TX<TL. More than 70%, 80%, 80-90% and 85% of the adsorbed protein still remained on LC, MC, TX and TL soil respectively, after being washed with water. Cry1Ab/Ac protein produced by *Bt* ssp. *kurstaki* can easily be adsorbed on the soil, while difficult to be desorbed.

FTP093

Development of a miniaturised screening-method for fungal mutants with enhanced production of specific natural compounds

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Fungi are well known as good producers of natural compounds. However, the potential of marine fungi to produce bioactive compounds is under investigated. To improve this knowledge, the EU-project MARINE FUNGI (EU FP, 265926) has set its focus on the isolation and characterisation of new anti-cancer compounds from marine fungi. To improve the production of the compounds or even to change the compound spectra diverse methods are used within the project. One good example for the enormous potential of marine fungi is a Scopulariopsis brevicaulis strain isolated from the marine sponge Tethya aurantium. The strain produces the cyclodepsipeptides scopularide A and B (1) and was selected for a molecular optimisation process using random mutagenesis by UV radiation. A challenge during this molecular optimisation process is the handling of the huge number of mutants, whose secondary metabolites are not easily detected, as e.g. by visual control or antibiotic activity determination. Hence, the specification of the secondary metabolites of each mutant strain is still a time and material consuming step. Therefore, a miniaturised screening method was developed. The established method covers a decreased cultivation volume, a fast extraction method and an optimised LC-MS analysis format. With this method a remarkable time reduction could be achieved and in addition a reduction of process deviation, important for the comparability of the screening results. With this method a mutant strain with enhanced production and a changed morphological growth behaviour compared to the wild type strain could be detected.

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FTP094

Persistence of DNA from genetically modified *Bt*-rice in four different paddy soils

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Rice is one of the major crops to feed the world's population. Due to high consumption of pesticides to protect the crops against insects, in recent times genetically modified (GM) rice was engineered to produce certain insect targeting toxins of the ubiquitous spore forming bacterium *Bacillus thuringiensis* (*Bt*) as protection. Although the toxins have a high selectivity and low toxicity against non-target organisms an important aspect of the environmental impact of GM rice is represented by the potential release of transgenic DNA into the soil by decaying crop residues especially roots.
It is known that transgenic DNA can persist in soil but there is virtually no information about the time of persistence. Although there is no report about

the introduction of GM rice DNA into soil microorganisms the potential threat of GM DNA in soil needs to be assessed.
The main objective of this research is to investigate the persistence of the DNA of the transgenic rice event TT51-1 by DNA extraction and quantitative real-time PCR (qPCR). A microcosm experiment was performed using four different paddy soils. Each soil was spiked with genomic DNA or plant material of TT51-1 rice in two different depths (0.5 cm and 10 cm) representative for oxic and anoxic layers in the soil profile. The total soil DNA was extracted by different methods comparing the suitability of the extraction assays. The quantity of extracted GM DNA was determined by qPCR. In addition, concentrations of Cry-toxins in the soil spiked with plant parts were analyzed by enzyme-linked immunosorbent assay (ELISA) showing the differences in Cry protein adsorption for the investigated soils.
Preliminary results indicate that the choice of DNA extraction method influences the detection, amount, integrity and purity of extracted DNA.

FTP095

Recycling of the compatible solute proline in the moderate halophile Halobacillus halophilus

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Proline is the major compatible solute in the moderate halophile *Halobacillus halophilus* at high salinities in the exponential growth phase. In the stationary phase proline is replaced by ectoine (1). Proline is degraded by proline dehydrogenase (ProDH) and pyrroline-5-carboxylate dehydrogenase (P5CDH) via pyrroline-5-carboxylate to glutamate. Both enzymes are encoded by two isogenes, of which only transcripts of prodh2 and p5cdh2 were shown to be upregulated at higher salinities (2). In cells grown at 3 M NaCl without external proline the activity of both ProDH and P5CDH was maximal in the stationary phase emphasizing their role in solute adaptation by degrading proline back to glutamate (2). Here, we further investigated the enzymes showing that the activity of ProDH increases with increasing salt concentrations and in a chloride-dependent manner. We will further investigate these findings on a molecular level by studying the enzyme activities of purified ProDH1 and ProDH2 to analyze the observed effects on a molecular level. Taken together, our findings suggest that the compatible solute proline is recycled by the enzymes of the proline degradative pathway to glutamate which makes ProDH and P5CDH responsible for the stationary phase-induced switch from proline to ectoine as compatible solute in *H. halophilus* (3).

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FTP096

LESA-HRMS - Surfaces analysis of bacterial signal molecules

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Communication between different microorganisms occurs particularly at their surface. To investigate intra- and innerorganismic communication in this zone, techniques have to be developed which detect the chemical signals directly at the surfaces.
Liquid extraction surface analysis (LESA) using TriVersa Nanomate technology is a promising and highly reproducible extraction infusion technique based on an automated chip-based nanoelectrospray ionization technique. Previously, we demonstrated that LESA combined with high-resolution mass spectrometry (HRMS) is a powerful tool to extract and detect thiazolyl peptide antibiotics from different Actinobacteria directly from agar plates (1).
We continued the survey and will present data concerning the variation of chemical signals depending on nutrient status and due to the physical presence of interacting organisms.

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FTP097

A toolbox for the heterologous gene expression in the phototrophic bacterium *Rhodobacter capsulatus*

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The genome era led to the identification of a tremendous number of genes which, however, need to be functionally expressed to make the respective proteins available for scientific studies and biotechnological applications. Consequently, novel and innovative expression systems are urgently needed. Thus, we developed a tailor-made bacterial expression system based on the phototrophic bacterium *Rhodobacter capsulatus* [1, 2]. Due to its metabolic versatility including chemoorganotrophic or photo(auto)trophic growth, this organism is especially suited for the expression of genes encoding heterologous membrane proteins and complex redox systems or the biosynthesis of hydrophobic metabolites [3]. Additionally, an appropriate and comprehensive set of broad-host-range plasmids was constructed. These broad-host range plasmids harbor different promoters, encode different antibiotic resistance cassettes and affinity tags and allow the comparative expression in *R. capsulatus* and a variety of Gram-negative bacterial standard expression strains including *Escherichia coli*. Recently, we have constructed a transfer and expression system (TREX) allowing the heterologous expression of large gene clusters and metagenomic libraries [4, 5].
Here, we present the expression of heterologous genes encoding soluble, secreted and membrane proteins in *R. capsulatus* as well as the applicability of the TREX system for the transfer and expression of large heterologous gene clusters.

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FTP098

Highly specific ectosymbionts of co-occurring closely related nematodes from the North Sea

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Ectosymbiotic bacteria are widespread on marine organisms but the specificity of these associations is poorly understood. Marine nematodes of the subfamily Stilbonematinae occur worldwide in coastal sediments. These worms carry a characteristic coat of sulfur-oxidizing ectosymbionts on their cuticle that they most likely acquire from their environment. To investigate the specificity of these symbiotic associations we studied closely related stilbonematid nematodes of the genus *Leptonemella* that co-occur in intertidal sandy sediments of the North Sea island of Sylt. Three *Leptonemella* species from Sylt have been described so far based on their morphology. Our phylogenetic analyses, based on the 18S rRNA gene of nematodes, collected from a single site on Sylt, revealed an unexpectedly high diversity of at least five distinct *Leptonemella* species. Our clone library analyses of the bacterial 16S rRNA gene and the ribosomal intergenic spacer region (ITS) of 22 worms and their symbionts showed that all five *Leptonemella* species host very closely related ectosymbionts belonging to a gammaproteobacterial clade of sulfur-oxidizing symbionts from marine nematode and oligochaete worms. We observed a high degree of host-symbiont specifity: each host species harbored a distinct 16S-ITS rRNA symbiont phylotype. Moreover, within a given host species we observed specificity at the individual level, with different individuals of the same *Leptonemella* 18S rRNA host group harboring a distinct ectosymbiont 16S-ITS rRNA subtype. Such remarkable specificity in very closely related co-occurring hosts and their symbionts has not yet been described and suggests the presence of co-evolved recognition and acquisition mechanisms that maintain these highly specific symbioses.

FTP099

Formation of the polyketide moiety of pyrroindomycins requires the synthesis of a tetramic ring and a few additional modifications

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Streptomyces rugosporus LL-42D005 produces pyrroindomycin A and its chlorinated form, pyrroindomycin B [1]. Pyrroindomycins are antibiotics active against Gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococci* strains [2]. Pyrroindomycins A and B are related to other compounds containing a tetramic or tetronic acid moiety spiro-linked to a cyclohexene ring. Some of these tetronic- and tetramic-containing compounds show very important biological activities such as tetronomycin [3], a polyether ionophoric antibiotic produced by *Streptomyces sp*. NRRL11266 that exhibits activity against Gram-positive bacteria or tetrocarcin A [4], an antibiotic with potent antitumor activity and versatile modes of action produced by *Micromonospora chalcea* NRRL 11289 or chlorothricin [5], a compound that inhibits cholesterol biosynthesis or kijanimicin [6], an antibiotic that shows also antimalarial activity.
Recently, the pyrroindomycin gene cluster was identified and its biosynthetic pathway has been proposed [7]. A type I PKS system and a modular NRPS are responsible for the formation of the backbone of the pyrroindomycin aglycone. Other genes such as *pyrE1-E3*, *pyrD3-D4* and *pyrI1* are also involved in its biosynthesis. Twelve genes, *pyrC1-pyrC12* that include 3 glycosyltransferases are responsible for the deoxytrisaccharide biosynthesis and the genes *pyrH* and *pyrK1-K3* for the attachment of pyrroloindole and its chlorination [7].
pyrE3, *pyrD4* and *pyrD3* have been identified which are suggested to be involved in formation of the tetramic ring and cyclisation of the polyketide moiety. The stop and start codons of *pyrD3* and *pyrD4* overlap, suggesting that they are translationally coupled and are parts of the same operon. In order to characterise the activity of these genes deletion mutants were created and their accumulated products analyzed. The resultant mutant strains neither produce pyrroindomycin A nor pyrroindomycin B, anymore. Instead, new compounds with no pyrroindomycin UV-spectrum were detected.

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FTP100

The monodechloroaminopyrrolnitrin 3-halogenase (PrnC) from pyrrolnitrin biosynthesis – a new type of flavin-dependent halogenase?

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The third enzyme in the biosynthesis of the antifungal antibiotic pyrrolnitrin, monodechloroaminopyrrolnitrin (MCAP) 3-halogenase (PrnC), catalyses the regioselective chlorination of MCAP in the 3-position of the pyrrole ring. PrnC is a flavin-dependent halogenase and its reaction mechanism is suggested to be similar to that of other flavin-dependent halogenases.
However, the amino acid sequence shows hardly any similarity to the amino acid sequence of flavin-dependent halogenases with known three-dimensional structures.
Additionally, PrnC is, besides the tryptophan halogenases, the only known flavin-dependent halogenase that catalyses the halogenation of a free substrate. Other known flavin-dependent halogenases catalysing the chlorination of a pyrrole moiety act on a substrate bound to a peptidyl carrier protein making elucidation of the 3-D structure of these enzymes, especially in the presence of substrate, rather difficult. Thus, the 3-D structure of PrnC is of high importance to understand how substrate specificity and regioselectivity are regulated in flavin-dependent halogenases.
Purification of PrnC in its active form is a challenging venture due to the incompatibility of conventional tags used and the high tendency of PrnC to form aggregates with itself and other proteins. Here, we report different purification strategies tested and established for purifying the halogenase. Based on protein models, we furthermore identified the amino acid serine 82 (S82) instead of the otherwise well conserved lysine residue (e.g. K79 in tryptophan 7-halogenase PrnA) as a putative amino acid essential for the chlorination of the pyrrole ring and show results on mutation studies. Moreover, when characterising PrnC via ICP-OES, we found copper to be incorporated into the enzyme, whose role has yet to be elucidated.

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FTP101

Influence of soil structure on growth and spatial distribution of soil microorganisms

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Soil microbes are well known for their capability to perform a wide range of activities in soil. Modern techniques like bioaugmentation or biosensors are used to enhance these microbial activities. Microbes occupy only 5% of the total available pore space in soil which demonstrates that the spatial arrangement of microorganisms in soil has a huge impact on their contact to target and on the way they interact. Most of the, studies to date have mostly involved destructive sampling of the soil, thereby destroying the characteristic of soil that underpins all processes, including access to carbon, movement of water, availability of air and diffusion of gasses. There is limited research on spatially characterising the distribution of indigenous or introduced bacteria at microhabitat scale. Studying the spatial pattern at microscale will inform us of factors that control microbial activities and therefore help in improving the predictive power of models for soil management. Therefore, this work brings together the quantification (X-ray) and visualization (microscopy) efforts to elucidate the role of soil structure on spatial distribution of bacteria in soil. A set of soil microcosms inoculated with gfp-tagged *P.fluorescens* strains under different treatments (bulk densities, water content and aggregate size) was set up. A subset of samples was fixed with paraformaldehyde, and impregnated with resin. GFP-signals of bacterial cells were visualised in thin sections of soil cores by epifluorescence microscopy to study the distribution and the soil structure was quantified by X-ray microtomography. Thus we merge X-ray microtomography and microscopy to visualise and quantify the impact of various soil physical properties on the spatial distribution of bacteria. The presented work is a significant step towards understanding how environmental change and soil management impact on bacterial diversity in soils. The future prospect of this research is to study the distribution and interaction between two species at microscale using CARD-FISH technique. This will assist in identifying experimental data to parameterise and further develop existing models for microbial dynamics.

FTP102

No magic bullets: The adverse effects of silver nanoparticles on microorganisms in waste water treatment plants

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Despite the promising developments and manifold novel applications of nanotechnology approaches, the increased use and release of nanoparticles into the environment raises major concerns. While the antimicrobial properties of silver nanoparticles are exploited in a large number of industrial products (*e.g.*, paints and varnishes, biocidal coatings, textiles, cosmetics) it is generally agreed that a major part will end up in waste water treatment plants (WWTPs) and eventually in the aqueous environment. To study the dose-response characteristics of potential toxic effects, activated sludge samples from the Giessen WWTP were treated with spherical Ag(0) nanoparticles (primary particle size distribution D90 <15 nm) and key functional parameters (*e.g.*, 0_2 demand, NH_4^+) were monitored over time. Concentrations ≥ 100 μg ml^{-1} revealed an acute toxicity on heterotrophic microbial populations. Size distribution of media-suspended Ag(0) nanoparticles was determined by Nanoparticle Tracking Analysis using a 532-nm laser and revealed an average particle size of 31 nm (D90 <45 nm). A protective effect on microbial growth of model organisms was observed by the addition of reference humic acids. Results from ICP-OES demonstrated that, with increasing humic acids concentration, the silver exposure of microorganisms was drastically reduced, most likely due to preferred sorption processes of the nanoparticles to humic acids. DGGE fingerprinting of amplified 16S rDNA fragments was applied to follow

temporal dynamics of the community structure in the presence of different Ag(0) nanoparticle concentrations. Substantial changes were observed in the range from 50 to 100 μg ml^{-1} Ag(0). The effects on key groups in activated sludge were studied using fluorescence *in situ* hybridization, imaged by confocal laser scanning microscopy and quantified with the *daime* tool. Further experiments aim to investigate the effects of nanosilver on a suite of toxicity endpoints including membrane integrity and intracellular esterase activity.

FTP103
Taxonomic and phylogenetic diversity of soil-related yeasts in Germany

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Soil yeasts are distributed globally. They occupy different ecological niches and provide important ecosystem services. Human activities, land management and climate change threats global biodiversity. Recent studies showed that long-term alteration of the native broadleaf vegetation (beech forests) within three UNESCO Biosphere Reserves in Germany substantially changed soil yeast communities [1, 2]. Forest management has little effect on biomass and total abundance values [2, 3] but influenced yeast diversity and community parameters [2].
In addition to the analyses of taxonomic diversity reported in previous studies [1, 2], we assessed phylogenetic diversity of soil yeast communities. Furthermore, we applied MLST approach to assess genetic variation of soil-related *Cryptococcus* (Tremellomycetes, Agaricomycotina) species and the four following loci were chosen for sequencing: rDNA SSU, LSU and ITS, and *RPB1*.
We isolated a total of 60 yeasts and 20% were new species. Out of these, *Cryptococcus terricola*, *Cr. terreus*, *Cr. aerius*, *Cr. podzolicus*, *Trichosporon dulcitum*, *T. porosum*, *Kazachstania piceae*, *Barnettozyma vustinii*, *Schwanniomyces castellii* and *Candida vartiovaarae* were the most frequent. We have observed that despite relatively low species richness in a given locality, yeast communities are much more diverse on larger geographic scales. This is mostly due to the fact that nearly half of the species were found in a single site only. Interestingly, yeast species typically showed greater intraspecific variability in regions where they were dominating.
Analysis of MLST data showed that the *RPB1* gene fragment showed the highest inter- and intraspecific variability in soil-related *Cryptococcus* species. Remarkably, in several instances the first universal fungal DNA-barcode (ITS, [4]) had a discriminatory power lower than LSU, suggesting that results derived from culture-independent soil surveys, which utilized ITS1 and ITS2 sequences may underestimate diversity of soil yeasts.

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GRV001
Living on the surface: Biofilm formation and surface motility of *Bacillus subtilis*

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Bacteria colonize surfaces in various ways. When growing on semi-solid surfaces, *Bacillus subtilis* shows swimming, swarming, and sliding motility depending on the agar concentrations. While swimming and swarming depend on the activity of flagella, sliding is a passive surface translocation and does not require an active motor. On medium with higher agar concentration *B. subtilis* forms architecturally complex colonies. Several regulators were described to affect motility behaviors or biofilm formation in *B. subtilis* [1].
Sliding motility and biofilm formation of *B subtilis* Natto are affected by the agar concentration used and depends on Spo0A, the global transcription regulator of sporulation. Spo0A integrates environmental signals related to starvation or stress conditions and activates various developmental pathways.
Examination of strains with reduced biofilm structure formation in *B. subtilis* resulted in the identification of *bslA* gene required for biofilm development [2]. BslA is a small secreted protein that forms a hydrophobic layer on the surface of *B. subtilis* biofilms and increases liquid repellency [3]. Transcription of *bslA* is regulated by several global regulators and shows a spatiotemporal expression pattern during the development of complex colonies. Interestingly, we detected altered expression of *bslA* gene next to the genes related to biofilm formation in our microarray experiments where we examined the sliding behaviour of *B. subtilis* Natto under sliding restrictive compared to permissive conditions (using *spo0A* mutant strain or higher agar concentration). Introduction of the *bslA* mutation into *B. subtilis* Natto reduced sliding motility. Further, our experiments show that the production of exopolysaccharide is also needed for sliding of *B. subtilis* Natto, while the protein component of the biofilm matrix, the amyloid fibers and the presence of flagella are not required for sliding.
BslA, therefore next to protecting the biofilm community against various stresses [4], contributes to surface spreading. Our results point to presence of shared regulators and genes for distinct surface-dependent growth of *B. subtilis*.

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GRV002
Spontaneous prophage induction in *Corynebacterium glutamicum*

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The era of genome sequencing revealed that phage DNA, in the form of prophages or phage remnants, is a common element in bacterial genomes. However, the impact on the physiology of the host is only known for a few well studied examples. The genome of the Gram-positive soil bacterium *Corynebacterium glutamicum* ATCC13032 contains three prophages of which the largest, named CGP3, accounts for 6% of the whole genome. Remarkably, recent studies demonstrated that CGP3 is spontaneously induced in a small fraction of wild type cells (1 to 3%) even in the absence of a specific stimulus [1]. Our current studies aim at identifying the trigger of this spontaneous prophage induction at the single cell level and to study the physiological consequences thereof.
For many prophages, the host SOS response serves as a specific stimulus for induction. To identify the trigger of CGP3 induction, we induced the SOS response in *C. glutamicum* by the DNA-damaging agent mitomycin C and analyzed the expression of CGP3 genes in a time-course DNA microarray experiment. Upon addition of mitomycin C the expression of the cellular SOS response as well as CGP3 genes was highly induced (100 to 1000 fold). The time-related expression pattern of CPG3 induction allowed us to estimate early and late genes involved in CGP3 induction. Interestingly, we identified *alpC*, encoding for an actin-like protein, among the early phage genes. First results suggest an important role of this cytoskeleton protein in replication and/or segregation of CGP3 DNA. Based on the results of the transcriptomic analysis, we constructed promoter fusions of early phage genes and *eyfp* and combined them with SOS reporter constructs (P_{recA}) to study the correlation of the cellular SOS response and prophage induction in single cells by flow cytometry. In fact, first experiments show a moderate correlation between the spontaneous up-regulation of the SOS response and spontaneous prophage induction in single cells under non-stressed conditions. Furthermore, we will present first live cell imaging studies using microfluidic chip devices, which enable us to monitor the dynamics of stress and prophage induction in real time.

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GRV003

Interactions between a sensor kinase and an ABC-transporter: a new signalling pathway in antimicrobial peptide detoxification modules

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Resistance of many Firmicutes bacteria against antimicrobial peptides is mediated by so-called detoxification modules that are comprised of a two-component regulatory system (TCS) and an ATP-binding-cassette (ABC) transporter. Strikingly, the transporters and TCSs have an absolute and mutual requirement for each other in both sensing of and resistance against their respective antimicrobial compounds: expression of the transporter is regulated by the TCS, yet the sensor kinase is unable to detect a stimulus in the absence of an active transporter. These findings suggest a novel mode of signal transduction where the transporter constitutes the actual sensor of antimicrobial peptides. Database searches revealed the wide-spread occurrence of such modules among Firmicutes bacteria, and parallel phylogenetic analysis showed that transporters and TCSs have co-evolved. Based on these findings, we hypothesize the formation of a sensory complex between both components. *In vivo* and *in vitro* approaches studying the bacitracin-resistance module BceRS-BceAB of *Bacillus subtilis* support this hypothesis by indicating direct physical contacts between the sensor kinase, BceS, and the transport permease, BceB. Furthermore, random mutagenesis of BceB showed that most loss-of-function mutations lie in the last three transmembrane helices, which may therefore contain the interaction interface between transporter and sensor kinase. Taken together, our results show that Bce-type ABC-transporters and TCSs have co-evolved to form self-sufficient detoxification modules against antimicrobial peptides, and suggest a novel signaling mechanism involving formation of a sensory complex between transporter and sensor kinase.

GRV004

Interaction of the fumarate sensor DctS with the binding protein DctB in *B. subtilis*

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The DctSR two-component system of *Bacillus subtilis* consists of the sensor kinase DctS and the response regulator DctR. It controls the expression of the aerobic C_4-dicarboxylate transporter DctA for the utilization of fumarate and succinate as carbon sources (1).
From several two-component systems it is known, that a third component plays an essential role as a cosensor of the sensor kinase. The DcuSR system of *E. coli*, for example, needs the transport proteins DctA and DcuB to regulate the activity of the sensor kinase DcuS (2). The DcuSR system of *E. coli* controls the expression of genes for fumarate respiration and of the aerobic C_4-dicarboxylate transporter DctA (3).
In *Bacillus subtilis*, the binding protein DctB plays an essential role in the DctSR system (1). Bacterial Two Hybrid Assays and *in vivo* crosslinking experiments show interaction of DctS with DctB, but no interaction with the transporter DctA.

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GRV005

The PpyS/PluR system of *Photorhabdus luminescens* mediates a novel way for cell-cell communication using pyrones

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Cell-cell communication via acyl-homoserine lactones (AHLs) is well studied in many Gram-negative bacteria. The prototypical communication system consists of a LuxI-type autoinducer synthase and a LuxR-type receptor, which detects the endogenously produced signal [1]. The symbiotic and entomopathogenic enteric bacterium *Photorhabdus luminescens* harbors the plenty of 39 LuxR-like receptors, but lacks any LuxI-type autoinducer synthase and is therefore unable to produce AHLs [2]. Here we show that one of these LuxR solos, named PluR, detects endogenously produced α-pyrones named photopyrones (PPYs) instead of AHLs as signal. Upon PPY-sensing, PluR activates expression of the adjacent *pcfABCDEF* operon, which leads to cell clumping. Testing different PPYs for *pcfA*-promoter activity identified PPYD as the most specific signal that is sensed by PluR with a concentration as low as 0.35 nM. Additionally the photopyrone synthase PpyS was identified as producer of the signaling molecule. The entire system consisting of PluR, the PluR-target operon *pcf* and PpyS, has been reconstituted in *E. coli* confirming the nature of this novel cell-cell communication circuit. Amazingly *E .coli* cells overexpressing the *pcf*-operon showed an increased pathogenicity against *Galleria mellonella* insect larvae concluding that PpyS/PluR-dependent cell-cell communication contributes to the high pathogenicity of *P. luminescens*. Indeed cell-density dependent PPY production could be observed in culture as well as in insects infected with *P. luminescens*. As LuxR solos are widespread in nature and other bacteria can also produce pyrones, our discovery extends the range of bacterial signaling molecules and raises novel fundamental questions about bacterial cell-cell communication in nature.

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GRV006

Intricate regulatory circuitry ensures the dependence of quorum sensing on nutrient limitation AND population density

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In the last several years, multiple studies have shown that the quorum sensing systems of many bacteria respond not only to population density, but also aspects such as nutrient limitation. However, until now, the mechanism(s) by which quorum sensing systems sense nutrient limitation is unknown.
We explore this question using the model plant symbiotic bacterium *Sinorhizobium meliloti*. The Sin quorum sensing system of *S. meliloti* initially responds positively to increasing population density with a massive production of acyl-homoserine lactones (AHLs), the inducer molecules which mediate quorum sensing. If population density continues to increase, an AHL-dependent negative feedback mechanism kicks in and shuts down AHL production. These attributes can be accounted for by three genes, which control transcriptional activation/repression of about 10% of the genes in the genome. *sinI* codes for the AHL synthase while *sinR* and *expR* code for inducer-binding transcription regulators, the so called LuxR regulators. In addition, *S. meliloti* contains multiple other genes predicted to code for orphan LuxR regulators, because they do not appear to be located in the vicinity of an AHL synthase. One of these, 877, strongly enhances AHL production in a specific temporal manner, resembling a burst in expression near the end of the logarithmic phase. This pattern is consistent during growth under nutrient limitations in carbon, nitrogen, or phosphorous. Microarray data, confirmed by fluorescence reporter assays reveals a subset of genes which are strongly altered in expression in response to either the deletion or over-expression of 877. Two of these genes are *sinR* and *phrR*, both of which are positively regulated by 877. However, *sinR* and *phrR* negatively regulate each other. The winner is determined by the presence or absence of AHLs.
These results suggest intricate regulatory logic allowing *S. meliloti* to engage in a quorum sensing lifestyle only if sufficient nutrients are available.

GRV007

Transcription activation of the promoters of mannitol utilization system in *Bacillus subtilis*

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Bacillus subtilis takes up mannitol via a phosphoenolpyruvate-dependent phosphotransferase system (PTS). Components of the mannitol utilization system ($EIICB^{Mtl}$, $EIIA^{Mtl}$, and mannitol 1-phosphate dehydrogenase) are encoded by the *mtlAFD* operon whose transcription is activated by MtlR [1, 2]. MtlR is a PRD-containing activator (PRD: PTS regulatory domains) and its activity is modulated by the phosphorylation/dephosphorylation of its regulatory domains, mainly PRDII and $EIIB^{Gat}$-like domains. *In vivo* and *in vitro* studies revealed that the phosphorylation of His 342 in the PRDII

domain by HPr(His15~P) and dephosphorylation of the Cys 419 in the $EIIB^{Gat}$-like domain by the $EIIA^{Mtl}$ phosphocarrier protein renders MtlR active [2-3]. Both of the promoters of *mtlAFD* (P_{mtlA}) and *mtlR* (P_{mtlR}) contain a σ^A-like structure [3]. So far, the MtlR-operator was deduced from the alignment of P_{mtlA} with P_{mtlR} of *B. subtilis* as well as P_{mtl} of *G. stearothermophilus* [1, 3]. Construction of the P_{mtlA}- P_{licB} hybrid promoters, where the putative MtlR-operator of P_{mtlA} was fused to the P_{licB} core elements, indicated the essential DNA sequence for the transcription activation of P_{licB} by MtlR. Likewise, *mtlR*-H342D C419A expressing the active form of MtlR was overexpressed in *E. coli* JW2409-1 (Δ*ptsI*) followed by purification of MtlR-H342D C419A with anion exchange chromatography. Using 5'-end Cy5-labeled P_{mtlA} and P_{mtlR} DNA fragments, electrophoretic mobility shift assay as well as DNA footprinting were performed to confirm the MtlR-operator *in vitro*. Finally, AT-rich sequences resembling the binding sites of carboxy terminal domains of the RNA polymerase α subunits (α-CTD) were found next to the MtlR-operator.

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GRV008

Integrative "Omics"-Approach Discovers Dynamic and Regulatory Features of the Bacterial Response to Singlet Oxygen

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Bacteria constantly face changing environments which may produce unfavorable conditions, usually termed stress. Bacteria therefore mount specific responses to finally achieve adaptation and survival. Stress responses were believed to be predominantly regulated at the transcriptional level. In the phototrophic bacterium *Rhodobacter sphaeroides* the response to singlet oxygen is initiated by alternative sigma factors [1]. However, post-transcriptional regulation by, e.g., small RNAs (sRNAs) was assumed to play a non-negligible role [2]. In addition, post-translational events have to be considered to gain a deeper understanding of how bacterial stress responses operate. To address this issue, we integrated three layers of regulation: (1) total mRNA levels at different time-points revealed dynamics of the transcriptome, (2) mRNAs in polysome fractions reported on translational regulation (translatome), and (3) SILAC-based mass spectrometry was used to quantify protein abundances (proteome). The singlet oxygen stress response exhibited highly dynamic features regarding short-term effects and late adaptation, which could in part be assigned to the sigma factors RpoE and RpoH2 generating distinct expression kinetics of corresponding regulons. The occurrence of polar expression patterns of genes within stress-inducible operons pointed to an alternative of dynamic fine-tuning upon stress. In addition to transcriptional activation, we observed significant induction of genes at the post-transcriptional level (translatome), while negative effects on protein abundance were rather triggered by degradation than by transcription or translation. Intriguingly, the SILAC approach identified 19 new open reading frames, which were partly validated on RNA level. We propose that integrative approaches as presented here will help to create multi-layered expression maps on the system level ("expressome") in various organisms and will help to understand how they adapt to alternating conditions.

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GRP001

Identification and Characterization of a Novel Transcriptional Repressor PhaR for the Steroid inducible Expression of the 3,17b-Hydroxysteroid Dehydrogenase Gene in *Comamonas testosteroni*

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Comamonas testosteroni (*C. testosteroni*) is able to catabolize a variety of steroids and polycyclic aromatic hydrocarbons, and might be used in the bioremediation of contaminated environments. 3,17b-Hydroxysteroid dehydrogenase (3,17b-HSD) from *C. testosteroni* is a key enzyme in steroid degradation. The genome of *C. testosteroni* ATCC11996 was sequenced in our previous work. In addition to the *3,17b-HSD* gene, a novel transcriptional repressor *PhaR* gene which locates 1747 bp upstream of the *3,17b-HSD* gene was found. PhaR knock-out mutants of *C. testosteroni* were prepared and shown to grow better than wild type *C. testosteroni* in the presence of 1 mM testosterone, 0.5 mM estradiol or 0.5 mM cholesterol in both SIN medium and 1:10 diluted SIN medium. After 1 mM testosterone induction 3,17b-HSD expression in the mutant was 2.5 times higher than in wild type *C. testosteroni*. Accordingly, PhaR is a repressor which controls 3,17b-HSD expression. Moreover, PhaR knock-out mutants grow at higher rates and produce more protein in the presence of steroids as carbon source. However, ELISA results showed that 0.5 mM estradiol and cholesterol could not induce *3,17b-HSD* gene expression in both wild type and mutant *C. testosteroni*. Probably, in addition to the *3,17b-HSD* gene, PhaR regulates some other genes which relate to steroid degradation. The gene coding for PhaR and 3,17b-HSD together with their promoter domains were cloned into plasmid pK18 and pUC19. *E. coli* HB101 was cotranformed with these plasmids. The results confirmed that PhaR is a repressor which might bind on a special 3,17b-HSD promoter domain (216 bp). A 2.521 kb DNA fragment which contains a putative promoter for the *RseB* and *3,17b-HSD* genes (without the *PhaR* gene) was cloned into plasmid pKPhaR11. The plasmid was transformed into HB101 (*E. coli*) and induced with testosterone. As a result, 3,17b-HSD expression was at a high levels but could not be further enhanced by testosterone. Taken together, PhaR knock-out mutants have stronger ability to degrade steroids than wild type *C. testosteroni* ATCC11996 and might therefore be used in bioremediation.

GRP002

NanR Regulates Amino Sugar Utilization in *Corynebacterium glutamicum*

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Amino sugars such as Glucosamine (GlcN) and N-Acetylglucosamine (GlcNAc) are valuable carbon and nitrogen sources for biotechnological processes. They are building blocks of the most abundant polymer chitin. Their efficient utilization in *C. glutamicum* contributes to sustainable value added and a more flexible feedstock for this organism. However, amino sugars are not a preferred carbon source. Growth rates on GlcN are low and GlcNAc cannot be utilized at all by *C. glutamicum*.
Recently we isolated a spontaneous mutant (M4) which is able to grow on GlcN as fast as on glucose as sole carbon source [1]. Microarray analyses revealed an upregulation of *nagA* (encodes N-acetylglucosamine-6P deacetylase) and *nagB* (glucosamine-6P deaminase). Both genes contitite an operon together with *scrB* (sucrose-6P-dehydrogenase). A single T>C point mutation located at position -18 with respect to the transcriptional start was responsible for higher transcription and higher enzymatic activity of NagB leading to improved growth on GlcN.
In the present work we describe the identification of the protein NanR that is involved in regulation of the *nag* genes. Electrophoretic mobility shift assays showed that purified NanR binds to the promoter region of the *nagAB-scrB* operon at exactly the same side where the mutation in M4 is located. Deletion of *nanR* leads to a higher promoter activity of *nagAB-scrB* and to an improved growth rate on GlcN showing that NanR is repressor of the *nagAB-ScrB* operon. Furthermore we showed that NanR regulates its own expression. Since *nanR* is located in a gene cluster comprising genes for uptake and degradation of sialic acid [2], the role of sialic acid as effectors for NanR is investigated.

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GRP003

Towards understanding global control and timing mechanisms of cereulide biosynthesis in *Bacillus cereus*

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The emetic lineage of *Bacillus cereus* produces cereulide, a cyclic dodecadepsipeptide, which is assembled by a non-ribosomal peptide synthetase (Ces NRPS). The biosynthesis operon (*ces*) is located on pBCE, a megaplasmid related to the pXO1 virulence plasmid of the close relative

Bacillus anthracis [1]. Cereulide is composed of alternating α-amino and α-hydroxy acids (D-*O*-Leu-D-Ala-L-*O*-Val-L-Val)$_3$ and toxic to human.
Although external stimuli influence cereulide synthesis, the process is tightly controlled and transcription peaks at the late-exponential growth phase [2]. As NRPS/PKS cluster expression is a highly coordinated process in the cell cycle not only of bacilli, but also of e.g. cyanobacteria and actinomycetes, our goal is to understand i) the mechanisms of timekeeping and ii) the global control of cereulide biosynthesis and its integration into the primary metabolism.
Likewise to the *B. anthracis* anthrax toxin genes, chromosomally located transcription factors were shown to be involved in regulation of the plasmid encoded *ces* genes [3, 4]. Nevertheless, virulence regulatory circuits in emetic *B. cereus*, integrating plasmid and chromosomal signals, are still poorly understood.
Using qRT-PCR, EMSAs, *lux* reporter studies, cell culture-based cytotoxicity assays, and a newly developed monoclonal antibody against the Ces NRPS, we show that the chromosomally encoded CodY protein acts as a transcriptional regulator on the *ces* promoter. By sensing the levels of GTP and branched-chain amino acids, CodY acts as a molecular timer to control cereulide formation and ties the cell´s nutritional status to this differentiation process. Additionally, a 2D-DIGE-based approach was used to study the influence of cereulide on the proteomic level.
Preliminary results indicate a strong dependence of cereulide biosynthesis on carbon balance and oxidative stress mechanisms. Further, complementary studies on transcriptional and translational levels suggest that *ces* gene expression is modulated by multi-layered realms of regulation.

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GRP004
The impact of sigma factors on global regulation of gene expression in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is a Gram-negative, rod-shaped and mono-flagellated bacterium known for its large metabolic diversity, its capability for adaptation and the high level of intrinsic antibiotic resistance. Furthermore, *P. aeruginosa* plays a major role as an important opportunistic and nosocomial pathogen that can cause acute and chronic infections affecting especially cystic fibrosis patients.
In *P. aeruginosa* a set of at least 24 sigma factors mediates the global regulation of gene expression by providing promoter recognition specificity to the RNA-polymerase holoenzyme. Under optimal condition, the housekeeping sigma factor RpoD drives most of the transcription, while stress conditions lead to a shift by inducing expression and activation of alternative sigma factors which in terms give rise to the transcription of specific subsets of genes (regulons) to cope with the challenging situation.
The aim of this project is to elucidate the impact of the major sigma factors RpoD, RpoE, RpoF, RpoH, RpoN, RpoS, PvdS and SigX on global gene expression in *P. aeruginosa*. To this end, reporter strains based on promoter-luxCDABE fusions were generated and the reporter activity was monitored in response to different growth conditions. Promoter activity studies were performed at the transcriptional as well as the post-translational level by analyzing promoters of the sigma factors themselves as well as of known sigma factor target promoters. Appropriate conditions regarding sigma factor activity were selected to perform RNA-sequencing as well as ChIP-sequencing approaches in order to identify the regulon and binding motif of each sigma factor. Finally, the phenotypic characterization of sigma factor mutant strains is in progress.

GRP005
The role of *atpI* in *Cupriavidus metallidurans*

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The *atp* operon encodes the 8 structural subunits a, c, b, δ, α, γ, β and ε of the F_1F_o-ATPase and subunit i, the only non-essential and non-permanent part of the complex (Gay and Walker, 1981, Solomon et al., 1989). F_1F_o-ATPase consists of a membrane integrated F_o part that translocates protons and a soluble F_1 part containing the catalytic sites for ATP synthesis from ADP and P_i using the electrochemical proton gradient (Capaldi and Aggeler, 2002; Okuno *et al.*, 2011).
The function of AtpI is still unknown although two roles have been proposed in different organisms: a chaperone-like function in c-ring assembly and a contribution to Mg^{2+} transport (Hicks *et al.*, 2003; Suzuki *et al.*, 2007). In *E. coli* the expression of the *atp* operon occurs from a promoter upstream of *atpI* (Kasimoglu *et al.*, 1996; Porter *et al.*, 1983).
In *Cupriavidus metallidurans* a large gap of 234 bp exists between *atpI* and *atpB*, the second gene of the *atp* operon. Initial analysis revealed a putative vegetative promoter localized in front of *atpI* and a putative stress dependent promoter in front of *atpB* as described in *Corynebacterium glutamicum* (Barriuso-Iglesias *et al.*, 2006). We are analyzing possible promoter regions in *C. metallidurans* to get better insights into the regulation of the *atp* operon.
Additionally, an interaction of the subunits i and c (encoded by *atpE*) has been observed in the past (Suzuki *et al.*, 2007). We present initial experiments to gain further insight into this interaction by using co-purification of native and modified *atpI*/*atpE* pairs.

GRP006
Regulation of Reductive Dehalogenase Gene Transcription in *Dehalococcoides mccartyi* strain CBDB1

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The strictly anaerobic bacterium *D. mccartyi* strain CBDB1 recognizes and dechlorinates a multitude of different chlorinated organic compounds in a process called organohalide respiration. Reductive dehalogenases (Rdh) catalyse the reduction of chlorinated compounds, while hydrogen functions as electron donor in the process. The 32 putative Rdhs enzymes of CBDB1 are encoded by *rdhAB* operons, with *rdhA* encoding the catalytic subunit and *rdhB* the membrane anchor. Genes encoding MarR-type regulators or genes encoding two-component systems neighbour the *rdhAB* genes, suggesting that their gene products might be involved in regulation of the *rdhAB* operon expression [1]. Until now only little is known about the regulation of reductive dehalogenases synthesis. We investigated the regulation of transcription of the two neighbouring *rdhA* genes *cbdbA1453* and *cbdbA1455*, which are preceded by the divergently oriented *marR* gene *cbdbA1456*. *Dehalococcoides* is not accessible to genetic analysis, therefore, regulation was studied in the heterologous host *E. coli*. Promoter-*lacZ* fusions were constructed for *cbdbA1453*, *cbdbA1455* and *cbdbA1456*, and these were introduced in single copy into the genome of *E. coli* MC4100 and they showed activity in the heterologous host. To address the question whether the MarR-type regulator CbdbA1456 acts as a repressor or activator of gene expression, *cbdbA1456* was cloned under the control of the arabinose-inducible P_{BAD} promoter. CbdbA1456 strongly repressed the *β*-galactosidase activity of the *cbdbA1455* promoter, and thus it probably acts as a repressor. To get information about the transcriptional regulation of *cbdbA1453* and *cbdbA1455* in strain CBDB1 we investigated transcription in response to 1,2,3- and 1,2,4-trichlorobenzene (TCB). RT-PCR analyses indicated that both genes were transcribed in the presence of 1,2,3-TCB, forming a polycistronic mRNA.

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GRP007
Structural and functional analyses of the membrane-integrated transcriptional activator CadC of *E. coli*: New insights into signal transduction

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The ToxR-like transcriptional activator CadC combines sensory function and DNA-binding activities in a single polypeptide. This type of regulators is characterized by an N-terminal cytoplasmic DNA-binding effector domain and a C-terminal periplasmic sensor domain separated by a single transmembrane helix. CadC regulates the expression of the *cadBA* operon at

low external pH and in presence of concomitantly available lysine. This in turn allows *E. coli* to adapt to acidic stress. We are interested in elucidating how the inner-membrane protein CadC is able to transduce signals across the membrane and subsequently activates transcription of the *cadBA* operon. Recently, it was demonstrated that the periplasmic domain of CadC is responsible for pH sensing [1,2]. However, almost nothing is known about the mechanism of signal transduction across the membrane to the cytoplasmic effector domain of CadC. A bioinformatics approach revealed a large unstructured loop of unknown function between the transmembrane helix and the DNA-binding domain. To investigate the role of the loop in terms of signal transduction, this part of the protein was gradually truncated or elongated. Resulting CadC variants activate *cadBA* expression independent of external pH, implying that the cytoplasmic loop plays an important role in transducing the signal to the DNA-binding domain. Further, the conformation and dynamics of the cytoplasmic domain of CadC is analyzed by NMR spectroscopy. The NMR data show that the N-terminal region adopts a tertiary structure while the C-terminus is intrinsically disordered and flexible in solution. NMR binding experiments confirm that the C-terminal region is not involved in DNA-binding.

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GRP008
Efflux pumps and TetR-like regulators in rhizobial interactions with plants

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Bradyrhizobium japonicum and *Sinorhizobium meliloti*, the nitrogen-fixing symbionts of soybean and alfalfa, respectively, possess a number of genes encoding efflux pumps of the major facilitator and RND superfamilies. Our goal is to identify and analyse efflux pumps that might be used by the rhizobia as defence against secondary plant metabolites. Previous microarray experiments, which were performed with both species, revealed that transcription of some of the systems is inducible by flavonoids [1-3]. For selected systems, regulation was analysed using reporter genes and electrophoretic mobility shift assays. Reporter gene assays showed that the genes are induced by a similar set of flavonoids as the nodulation genes, however, no *nod* box-like sequences, which are typical for nodulation genes, were found to be present in the putative promoter regions. Therefore, this regulation seems to work independently of the regulatory pathways involved in the induction of nodulation genes by flavonoids. Adjacent to the studied efflux pump genes and in divergent orientation, proteins belonging to the TetR regulatory family are encoded. The genes were heterologously expressed in *Escherichia coli* and the TetR-like proteins were purified. Electrophoretic mobility shift assays showed that they bind to the intergenic regions. Using oligonucleotides, we found that palindrome-like sequences within the intergenic regions are sufficient and required for binding. This suggests that the regulatory proteins act as repressors that are released from their operator sites upon the interaction with flavonoids leading to an increased efflux. In accordance with that, we found that some flavonoids decrease the affinity of the proteins for their binding sites. Currently, we are testing mutants for their symbiotic behaviour. Our studies will further elucidate how rhizobia might overcome intrinsic host defences.

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GRP009
Regulation of acetoin formation by the transcriptional regulator AlsR of *Bacillus subtilis*

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Bacillus subtilis forms acetoin under anaerobic fermentative growth conditions and as a product of the aerobic carbon overflow metabolism. Acetoin formation requires α-acetolactate synthase and acetolactate decarboxylase, both encoded by the *alsSD* operon. The *alsSD* expression is dependent on the transcriptional regulator AlsR which belongs to the LysR family of transcriptional regulators. AlsR is composed of two domains: an N-terminal DNA binding domain with a winged HTH motif and a C-terminal regulatory domain which is involved in co-inducer binding and oligomerization. To identify functional relevant amino acid residues we mutagenized the *alsR* gene by site directed mutagenesis and analysed the *in vivo* function. AlsR activity was monitored by ß-galactosidase activities derived from an AlsR-dependent *alsS-lacZ* reporter gene fusion. In addition, we produced and purified the AlsR mutant proteins and analysed the *in vitro* binding ability by EMSA analyses. The amino acid exchange from serine at position 100 of AlsR to alanine inactivated the AlsR protein and transcriptional activation *in vivo* and *in vitro* was abolished. Moreover, the AlsRS100A mutant protein showed an altered DNA/protein complex pattern compared to the wild type protein. In EMSA analysis the wild type AlsR protein formed 3 different migrating complexes, whereas the AlsRS100A mutant protein is no longer able to form the slowest migrating complex III. Deletion of 5 bp and thereby reducing the distance between RBS and ABS abolished complex III formation. In addition, the *alsS*-promoter with the same 5 bp deletion was unable to activate *lacZ* expression. Therefore, we assumed that complex III is the transcriptional active form. We postulated a model of AlsR mediated regulation where effector-bound AlsR is forming an active complex of AlsR octamers bending the DNA (1).

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GRP010
A Hydrophobic Interface Required for Homo-dimerization and Signal Transfer in PAS_C of *Escherichia coli* DcuS.

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Metabolic regulation of aerobic and anaerobic C_4-dicarboxylate utilization of *E. coli* is effected by the two-component system DcuSR, consisting of the membrane-embedded sensor histidine kinase DcuS and the response regulator DcuR. Binding of C_4-dicarboxylates results in autophosphorylation of a conserved histidine residue. The cytoplasmic PAS_C domain transfers the signal from the transmembrane region to the kinase.
24 crucial residues within PAS_C were identified by combining error-prone-PCR based random mutagenesis with the site-directed substitution of amino acids that were relevant for structural reasons. Variants of DcuS were classified by their effect on DcuS functionality into ON and OFF. Most of the ON-mutations were localized in a hydrophobic cleft that was important for DcuS dimerization. Introducing charged residues in that region relieved DcuS dimerization, demonstrating the significance of this region for signal transmission in PAS_C.

GRP011
Combined oxygen and nitrate sensing by the *Staphylococcus carnosus* NreABC three-component system

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The NreABC three-component system is required for oxygen and nitrate sensing and activates nitrate respiration in *Staphylococcus carnosus* [1]. In active state the sensor kinase NreB contains an O_2 sensitive $[4Fe\text{-}4S]^{2+}$ cluster which is converted by O_2 to a $[2Fe\text{-}2S]^{2+}$ cluster followed by complete degradation and formation of inactive FeS-less apoNreB [2]. Phosphotransfer from NreB to the regulator NreC in the absence of oxygen leads to the expression of *narGHJI* encoding nitrate reductase [1].
NreB and NreC are encoded in one operon (*nreABC*) together with the protein NreA. NreA is necessary for nitrate sensing and deletion of *nreA* causes aerob derepression of *narG* expression. The crystal structure of NreA revealed a GAF domain protein with a nitrate binding pocket liganding nitrate. In vitro, NreA inhibits NreB autophosphorylation and inhibition is relieved when NreA binds nitrate.
NreA is a nitrate sensor which modulates the autophosphorylation activity of the oxygen sensor NreB.

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GRP012

Looking for natural competence in *Bacillus megaterium*

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The selective inactivation of chromosomal genes is essential for the rational optimization of microbial hosts in the sense of eliminating bottlenecks, thereby elevating the quality and quantity of recombinant protein production. One easy and fast way of genetically manipulating some bacterial hosts is provided by their natural competence. Detailed studies on this mechanism in *Bacillus subtilis* led to the identification of many genes involved in its regulation and the DNA translocation machinery [1]. Due to the recently available genome data of the biotechnologically interesting bacterium *Bacillus megaterium*, detailed *in-silico* analyses were performed elucidating its physiological capabilities [2]. Thereby, homologues to all genes known to be essential for DNA-uptake and recombination during natural competence were annotated which in principle should enable this organism to develop natural competence. As this state is strictly regulated in *B. subtilis* as well as many other bacteria, depending on growth phase, cell density and growth conditions, the question remains under which conditions *B. megaterium* develops natural competence, if it does at all.
Here, the expression of several putative competence associated genes from *B. megaterium* was investigated using different reporter systems as well as transcriptome analysis under different conditions, such as growth medium, antibiotics and other supplements. Hereby, first ideas about the conditions leading to the induction of the competence regulon could be identified.
Furthermore, to elucidate the regulatory mechanisms underlying the expression of natural competence associated genes in this organism, different regulated *B. megaterium* gene libraries were created. These allow for the identification of possible regulators involved in natural competence by using reporter-systems in which the expression of competence associated genes is screened for. Possible regulators identified by this approach could be further investigated with regards to their ability to induce natural competence, thereby giving a better understanding of the regulation of this mechanism.

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GRP013

The individual roles of MeqR1 and MeqR2, two PaaX-like regulators, in the transcriptional control of 2-methylquinoline degradation by *Arthrobacter* sp. Rue61a

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Arthrobacter sp. Rue61a is a Gram-positive soil bacterium which is able to utilize 2-methylquinoline as source of carbon and energy. Its genome consists of a circular chromosome (4.7 Mbp) and a circular (231.5 kbp) and a linear plasmid (113 kbp). The genes required for the conversion of 2-methylquinoline to anthranilate are located on the linear plasmid pARUE113 and are clustered in two divergently oriented “upper pathway” operons (ORFs 3-6 and ORFs 7-11). A third operon (ORFs 19-23) codes for enzymes involved in anthranilate degradation, which exclusively occurs via coenzyme A (CoA)-thioester intermediates [1].
Two open reading frames (ORF16 and ORF24) flanking the lower pathway operon code for two PaaX-like transcriptional regulators MeqR1 and MeqR2 (2-methylquinoline regulator 1 and 2). For MeqR1 and MeqR2, we determined a highly conserved palindromic binding site (5´-TGACGNNCGTcA-3´) upstream of all three catabolic operons and the *meqR2* gene. Anthraniloyl-CoA was identified as specific derepressing effector for both regulators by *in vitro* assays.
Using qRT-PCR, the expression levels of the catabolic operons, *meqR1*, and *meqR2* were determined in cells of the Δ *meqR1* and Δ*meqR2* mutant strains grown on glucose or anthranilate, and compared to those of the wild-type strain. The results showed a strong effect of MeqR1 on the lower pathway operon, whereas MeqR2 mainly acts on one of the upper pathway operons (ORFs 7-11). Interestingly, the qRT-PCR data revealed that additional regulatory elements superimpose the MeqR specific regulation.
For *in vivo* studies, the promoter regions of the catabolic operons were transcriptionally fused to the *luxCDABE* genes. Each promoter-*lux* fusion was combined with either the *meqR1* or *meqR2* gene under the control of a rhamnose-inducible promoter. The reporter systems will be analyzed in a heterologous background (*Escherichia coli*) as well as in strain Rue61a and its ΔpARUE113 mutant to elucidate the individual roles of MeqR1 and MeqR2 in the regulation of 2-methylquinoline degradation.

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GRP014

Cooperation of the two-component systems ChrSA and HrrSA involved in heme-dependent gene regulation in *Corynebacterium glutamicum*

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Heme is an important cofactor for proteins of diverse functions and is used as an alternative iron source by many bacterial species.
Previous studies revealed that the two-component system (TCS) HrrSA plays an important role in the control of heme homeostasis in *Corynebacterium glutamicum*. The response regulator HrrA functions as an activator of genes involved in heme utilization, especially for expression of the heme oxygenase (*hmuO*). Recently, we could demonstrate that the homologous TCS ChrSA represents a second system involved in heme-dependent control of gene expression and is crucial for conferring resistance towards high levels of heme. Transcriptome analysis, *in vitro* DNA-protein interaction studies and promoter fusion assays indicated that ChrSA directly activates the divergently located operon *hrtBA*, encoding a putative heme exporter. To determine under which conditions the systems are active and influence expression of target genes we systematically recorded target promoter activities (P_{hrtB} and P_{hmuO}) under varying heme and iron concentrations. First results confirmed that both systems strictly depend on the presence of heme, but in contrast to ChrSA, which is not influenced by iron, HrrSA is mainly active under iron limiting conditions.
The coexistence of two TCS´s in one species, both involved in heme-dependent gene regulation, raises the question how and on which levels these systems interact with each other. Up to now EMSA and promoter fusion assays delivered evidence for a reciprocal cross-regulation of HrrA and ChrA at the transcriptional level. Furthermore, systematic mutational analysis of the two systems suggested cross-talk *via* phosphotransfer between HrrSA and ChrSA. The current model is premised on the assumption that this highly intertwined network is required for the balance between heme detoxification and use as an alternative source of iron.

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GRP015

The aconitase of *Bacillus subtilis*: fine-tuning the entrance into the citric acid cycle

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The citric acid cycle plays a central role in the metabolism of most organisms as it provides the cell with energy and cellular building blocks. Especially in bacteria the pathway importantly contributes to their diverse metabolic abilities.
In the Gram-positive soil bacterium *Bacillus subtilis*, the regulation of the citric acid cycle is mainly exerted on the transcriptional control of the first two enzymes, citrate synthase and aconitase. The expression is controlled by the global transcription regulators CcpA and CodY and also by the citric acid cycle specific transcription regulator CcpC [1]. In the presence of a preferred carbon source the expression of citrate synthase is repressed by CcpA. Moreover in the presence of a preferred carbon and good nitrogen source the transcription of citrate synthase and aconitase is additionally repressed by CcpC. When the intracellular pools of GTP and branched-chain amino acids are high *citB* is further repressed by CodY [1]. Together the interplay of the transcription factors leads to a strong repression of the citric acid cycle branch under conditions of good nutrition supply.
Here we demonstrate a novel mechanism that controls the entry into the citric acid cycle by the bifunctional protein aconitase. On the one hand the aconitase is active as metabolic enzyme in the citric acid cycle and on the

other it can modulate translation of certain mRNAs under iron-limiting conditions at the post-transcriptional level [2]. In an aconitase mutant of *B. subtilis* the mRNA of citrate synthase was found to be stabilized and gel-shift experiments revealed a direct binding of aconitase to the *citZ* 5'UTR. Thus, under iron-limiting conditions the aconitase destabilizes the mRNA of citrate synthase and reduces the level of citrate synthase protein in the cell.

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GRP016

Characterization of some key players in the genetic switch of temperate *Streptococcus thermophilus* phage TP-J34

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In the sequenced lysogeny module of the temperate *Streptococcus thermophilus* phage TP-J34 all but four adjacent orfs point in one direction (Neve et al., 1997). They are separated from the potential lytic cycle-promoting genes *cro* and *ant* by a genetic switch region, which contains two divergently orientated promoters and putative operator sites. In order of transcription, they code for the putative repressor Crh, the putative metalloproteinase Orf3, the superinfection exclusion (sie) mediating lipoprotein Ltp (Sun, 2006), and the putative integrase. The genes *crh* and *orf3* are transcribed together as a polycistronic mRNA starting from promoter P_2. As a part of the genetic switch, the putative repressor Crh (cI-repressor homologue) is supposed to be essential for the establishment of lysogenization by suppressing lytic genes. The *crh* gene product has been overproduced by heterologous expression in *E. coli* to perform electromobility shift assays with purified protein. Four operator sites in the intergenic regions betweeen *crh* and *cro* and between *cro* and *ant*, respectively, could be verified by competition assays using synthetic oligonucleotides. Applying glutaraldehyde as crosslinking reagent, oligomerization - particularly formation of Crh dimers, tetramers and obviously even higher organised multimers in solution - has been proved. Knock out experiments of the adjacent *orf3* gene revealed an important role of the putative metalloproteinase in induction of the lytic cycle. Studies on the interaction between *crh* and *orf3* gene product indicated that Orf3 prevents/inhibits binding of the Crh repressor to his operator sites. Cro, the putative repressor of lysogenic genes, showed binding activity in the intergenic region between *crh* and *cro*. It probably blocks promoter P_2 upstream of *crh*. This would explain the antagonistic role of Cro in relation to Crh. Nevertheless, the exact processes in the genetic switch, causing either a lytic or a lysogenic development of TP-J34, still remain unclear.

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GRP017

The NreA protein functions as a nitrate sensor in nitrate regulation system of *Staphylococcus carnosus*

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Staphylococcus carnosus is a facultative anaerobic bacterium that is able to grow by aerobic and by nitrate respiration. For initiation of nitrate respiration the nitrate regulation system (Nre) is necessary [1], and the NreABC proteins are encoded in one operon (*nreABC*). The sensor kinase NreB senses oxygen directly [2]. NreBC activates the expression of genes of nitrate respiration only under anaerobic conditions [1] and nitrate further increases the expression level of the genes. Now, the role of the third component NreA was characterized using structural and functional analysis. The structure of an NreA·NO_3 complex was solved and NreA revealed a GAF fold with a nitrate ion in the binding pocket. Mutations in NreA that were designed to abolish nitrate binding affected the nitrate dependent induction of genes.

The experiments suggest that NreA serves as the nitrate receptor in the nitrate regulatory system of *S. carnosus*.

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GRP018

Program of quorum sensing dependent expression in the *Sinorhizobium meliloti* Sin/ExpR regulon

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The Sin quorum sensing (QS) system of *S. meliloti* regulates many genes, including those controlling motility and exopolysaccharide production. The system consists of an *N*-acyl-homoserine lactone (AHL) synthase, SinI, at least two LuxR-type regulators, SinR and ExpR. SinR appears to be independent of the presence of AHLs for its control of *sinI* expression, while ExpR is almost completely dependent upon AHLs. In this study, we focus on characterising the ExpR/AHL regulon by identifying novel ExpR binding sites and their importance for regulation of promoter activity. We confirm 7 previously detected ExpR-DNA binding sites and use the consensus sequence to identify another 26 sites, some of which regulate genes previously unknown to be members of the ExpR/AHL regulon. The activity of promoter regions containing ExpR sites were titrated against AHL levels, with varied outcomes in AHL sensitivity. The data suggests a type of quorum sensing program whereby each promoter has its own response range with respect to AHL concentrations. Promoter activity varied with AHL concentration up to 5000 nM, although most promoters respond within a more restricted range. Generally, promoters responding positively to AHLs are more sensitive, although three promoters displayed an exception to this. Lastly, we provide evidence that several aspects of ExpR binding sites appear to determine their biological function, such as the DNA sequence within the binding site, and the location of the ExpR binding site with respect to the transcription start, which determines whether ExpR/AHLs represses or activates promoter activity.

GRP019

Comparison of hightroughput techno-logies in the pathogenic fungus Aspergillus fumigatus *reveals novel insights into the genome*

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The filamentous fungus *Aspergillus fumigatus* has become the most important airborne fungal pathogen. This fungus can cause a life-threatening disease, invasive aspergillosis (IA), in immuno-compromised patients. Patients that suffer from IA still have a low life expectancy.
The developed high-throughput transcriptome and proteome technologies, coupled with the ability of creating and analysing huge databases, has paved the way for system biology's "Golden Age". The "omics" era provides a multitude of inputs that need to be integrated and assessed. Nowadays, a big effort is made to standardise data in order to generate datasets that can be used to investigate and compare pathways and gene responses involved in many processes including pathogenicity. We therefore evaluated the potential of paired-end RNA-Seq for investigating the regulatory role of the central mitogen activated protein kinase MpkA. As previously reported, this kinase acts in the *A. fumigatus* cell wall integrity signalling pathway and it is essential for maintaining an intact cell wall in response to stress .
Transcriptome analysis revealed that 70.4% of the *A. fumigatus* genome was found to be expressed and that MpkA plays a significant role in the regulation of many genes involved in cell wall remodelling, oxidative stress, iron starvation response, and primary and secondary metabolism.

The obtained data were further processed to evaluate the potential of the RNA-Seq technique. We comprehensively matched up our data to published transcriptome studies and were able to show an improved data comparability of RNA-Seq experiments independently of the technique used.
We additionally checked Transcript Active Regions (TARs) for open reading frames. Since this approach is error prone, we employed *ab initio* gene prediction combined with mRNA-Seq data to improve the quality of gene prediction. This evidence-based gene prediction has the advantage over the classic *ab initio* prediction of finding gene structures by making use of the mRNA-Seq expression profiles. This combination incorporates intron-junction information derived from intron-spanning reads and allows the prediction of not translated regions (UTR) in a systematic manner. By using this approach, it was possible to identify 185 new transcripts coding for putative proteins.

GRP020
The master regulator HexA and phenotypic heterogeneity of *Photorhabdus luminescens* cell populations

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Photorhabdus luminescens is a bioluminescent enteric bacterium that lives in a mutualistic association with soil nematodes and is in turn highly pathogenic towards insects. The bacterium exists in two different forms designated as the primary and the secondary variant [1]. Both variants are genetically identical but differ in many physiological and morphological traits. After a prolonged cultivation, single cells undergo phenotypic switching and single primary cells convert to the secondary variant. One of the most dominating differences between both variants is that only the primary cells can support nematode growth and development. Secondary cells are believed to be better adapted to a free-living stage when they remain in the insect cadaver after nematode emergence.
One master regulator being involved in the switching process is HexA, a LysR-type transcriptional regulator, which is supposed to act as a repressor of primary-specific genes in secondary cells [2]. Here we examined the function of HexA in regulating bistable gene expression and therefore phenotypic switching of single cells. Investigation of P_{hexA}-*mcherry* promoter activity at the single cell level in primary cells by time-lapse fluorescence microscopy showed a heterogeneous distribution of P_{hexA} activity. In a growing culture, single cells developed a strong P_{hexA} activity whereas others showed no or only weak activity. Cell populations overproducing HexA simultaneously showed a drastic decrease in bioluminescence. As a dark phenotype is characteristic for secondary cells, it is assumed that those cells with high P_{hexA} activity later convert to secondary cells. By using a bacterial two-hybrid screening approach, three potential proteins that interact with HexA have been found. Whether these proteins act up- or downstream of HexA, and whether they transmit signals into the switching process will be investigated in the future.

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GRP021
Determinants of phenotypic heterogeneity in the broad host range symbiotic α-proteobacterium *Sinorhizobium* sp. NGR234

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Phenotypic heterogeneity is an essential parameter for the persistence of bacterial populations in often-rapid changing environments. The knowledge on molecular mechanisms that are linked to bacterial heterogeneity is rather poor and only few genes are known that are determinants of heterogeneity [1].
We are analyzing the basic factors of phenotypic heterogeneity in the plant symbionts *Sinorhizobium* sp. NGR234 and its close relative *S. fredii* USDA257. NGR234 and USDA257 are so called broad host range strains. NGR234 is able to establish a nitrogen fixing symbiosis with more than 112 plant genera and USDA257 with about 79. The microbes share a high degree of syntheny in their genomes [2-4] and thus are ideal model microbes for studies with relevance to host range. We postulate that host range and plant infection are influenced by levels of phenotypic heterogeneity. To verify this hypothesis we measure the levels of phenotypic heterogeneity in laboratory and rhizosphere studies. Thereby we focus on genes linked to the synthesis and degradation of cell-cell communication signal molecules (N-AHLs) and genes linked to the built up of secretion systems. With the help of promoter fusions of the main autoinducer synthases and secretion system genes we are analyzing the transcription levels of the corresponding genes on a single cell level. Initial tests using *traI*- and *ngrI*- promoter fusions suggest that phenotypic heterogeneity depends not only on the presence of autoinducers but is also influenced by plant root exudates.

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GRP022
The *Janthinobacterium* sp. HH01 genome encodes a homologue of the *V. cholerae* CqsA and *L. pneumophila* LqsA autoinducer synthases

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Janthinobacteria commonly form biofilms on eukaryotic hosts and are known to synthesize antifungal compounds [1]. *Janthinobacterium* sp. HH01 was recently isolated from an aquatic environment and its genome sequence was established. The genome consists of a single chromosome and reveals a size of 7.10 Mbp. Approximately 80% of the 6,162 coding sequences (CDSs) present in the HH01 genome could be assigned to putative functions. Interestingly, the genome of HH01 apparently lacks the N-acylhomoserine lactone (AHL)-dependent signaling system and the AI-2-dependent quorum sensing regulatory circuit of many Gram-negative bacteria. Instead it encodes a homologue of the *Legionella*- and *Vibrio*-like autoinducer synthase gene (*lqsA*/*cqsA*) [2,3], which we designated *jqsA*. The *jqsA* gene is linked to a cognate sensor kinase (*jqsS*) and is flanked by the response regulator *jqsR*. Here we show that a *jqsA* deletion has strong impact on the regulation and expression of the violacein biosynthesis genes in *Janthinobacterium* sp. HH01. We also show that the *jqsA* deletion mutant can be functionally complemented with the *cqsA* and *lqsA* genes. Therefore it might be useful for the detection of homologues from other Proteobacteria and for studying the importance of the CqsA/LqsA/JqsA regulatory networks in the background of a non-pathogenic microbe. This appears tempting, since HH01 is genetically accessible and mutations can be generated with relative ease in this microbe. Furthermore it is our interest to determine the structure of the postulated JAI-1 signaling molecule.

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GRP023
Impaired nitrate/nitrite assimilation in *S. tsukubaensis*

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Streptomyces usually grow in nutritional limiting environment often lacking essential elements for growth. *Streptomyces coelicolor*, a model organism for studying the regulation of nitrogen metabolism exhibits a specific regulatory network. In this control system the OmpR-like transcriptional regulator GlnR plays a central role by controlling the transcription of at least 14 genes, 9 of which are directly implicated in the nitrogen assimilation. We identified genetic components of the nitrogen metabolism and regulation in the genome of *S. tsukubaensis*. Following key components involved in the nitrogen assimilation, up-take and regulation were found: two regulatory genes *glnR* and *glnRII;* post-translational regulatory components: *glnK, glnD* and *glnE;* ammonia up-take and assimilation genes: *amtB*, *glnA, glnII, gdh, glnA2* and *glnA4* and urea assimilation genes: *ureABC*. Surprisingly, genes encoding proteins involved in the assimilation of nitrate and its regulation like: *nnaR* (transcriptional regulator), *narB*/*nirBD* (nitrate/nitrite reductases) and *narK1* (nitrate transporter) are missing in the genome. Only one nitrate reductase gene *nasA* and one nitrate/nitrite symporter *narK2* were

found in the genome (both with rather low similarity to *S. coelicolor* genes). This result strongly suggests that the nitrate assimilation might be impaired in this strain. Growth studies with *S. tsukubaensis* wild-type on different nitrogen sources showed retarded growth on defined solid Evans medium supplemented with 100 mM nitrate. Since the nitrate/nitrite assimilatory pathway seems to be impaired and no assimilatory nitrite reductase was found in the genome - this strain is probably not able to reduce the toxic nitrite to ammonia what can cause a toxic effect resulting in retarded growth. To improve the nitrate/nitrite assimilation the *nirBD* gene encoding the nitrite reductase from *S. coelicolor* was heterologously expressed.

GRP024

A thermophile under pressure: Transcriptional analysis of the response of *Caldicellulosiruptor saccharolyticus* to different H_2 partial pressures.

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Caldicellulosiruptor saccharolyticus is an extremely thermophilic, gram-positive anaerobe which ferments a broad range of substrates to mainly acetate, CO_2, and hydrogen gas (H_2). Its high hydrogen-producing capacity make this bacterium an attractive candidate for microbial biohydrogen production. However, increased H_2 levels tend to inhibit hydrogen formation and lead to the formation of other reduced end products like lactate and ethanol [1]. To investigate the organism's strategy for dealing with elevated H_2 levels and to identify alternative pathways for reductant disposal, the effect of the hydrogen partial pressure (P_{H2}) on fermentation performance was studied [2]. For this purpose cultures were grown under high and low P_{H2} in a glucose limited chemostat setup. Transcriptome analysis revealed the up-regulation of genes involved in the disposal of reducing equivalents under high P_{H2}, like lactate dehydrogenase and alcohol dehydrogenase as well as the NADH-dependent and ferredoxin-dependent hydrogenases. These findings were in line with the observed shift in fermentation profiles from acetate production under low P_{H2} to a mixed production of acetate, lactate and ethanol under high P_{H2}. In addition, differential transcription was observed for genes involved in carbon metabolism, fatty acid biosynthesis and several transport systems. The presented transcription data provides experimental evidence for the involvement of the redox sensing Rex protein in gene regulation under high P_{H2} cultivation conditions. Overall, these findings indicate that the P_{H2} dependent changes in the fermentation pattern of *C. saccharolyticus* are, in addition to the known regulation at the enzyme/metabolite level, also regulated at the transcription level.

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GRP025

Regulatory features of the phage shock protein A (PspA) and a newly identified regulator of the phage shock system

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The Psp-system (phage shock protein) is currently thought to be a membrane stabilizing system that is conserved in many bacteria as well as in plant chloroplasts. In *E. coli,* where it is best studied, the system comprises 7 known proteins (PspA-G), which are - with the exception of PspF - extensively produced during extreme membrane stress conditions like addition of 10% ethanol or severe heat stress. The four proteins PspF, PspA and PspB/C seem to be the key players of the system: PspA is a coiled-coil protein and the central effector protein of the system, while PspB/C are integral membrane proteins and PspF a cytoplasmic regulatory AAA+ ATPase. PspA has a dual role, as it is capable of a) formation of large oligomeric complexes at the membrane, probably aided by the membrane proteins PspB/C and b) binding of the operon's activator protein PspF to down-regulate production of the Psp proteins. We will present our work on the regulation of the Psp-system, especially our recent findings concerning remarkable features of the regulatory PspA/F complex that allow us to silence the Psp system in *E. coli*. We will also present a newly identified - and to our knowledge, first - Psp-regulating protein in *E. coli* that is not part of the PspF regulon, leading to new insights into the complex mechanism of regulation and the potential role of the Psp system.

GRP026

Novel factors involved in the rapid degradation of the most unstable protein in *Bacillus subtilis*

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The Gram-positive model bacterium *Bacillus subtilis* contains two glutamate dehydrogenase-encoding genes, *rocG* and *gudB*. While the *rocG* gene encodes the functional GDH, the *gudB* gene is cryptic (*gudB*^{CR}) in the laboratory strain 168 due to a perfect 9-bp direct repeat that renders the GudB enzyme inactive and unstable (1). Although constitutively expressed the the GudB^{CR} protein can be hardly detected in *B. subtilis* as it is rapidly degraded (2, 3). Its high instability qualifies GudB^{CR} as a perfect model substrate for studying protein turnover in *B. subtilis*. We have developed a visual screen to monitor the GudB^{CR} stability in the cell by using a GFP-GudB^{CR} fusion. Using fluorescent microscopy we found that the GFP protein is simultaneously degraded together with GudB^{CR}. This allows us to analyze the stability GudB^{CR} in living cells. By combining the visual screen with a transposon mutagenesis approach we looked for mutants that show an increased fluorescence signal compared to the wild type indicating a stabilized GFP-GudB^{CR} Fusion. We found four different transposon insertions affecting the oligopeptide ABC transporter Opp which is involved in sporulation and competence development (4, 5). Also, we found one transposon insertion in the *mcsB* gene encoding an arginine kinase (6). Interestingly, the GudB^{CR} protein was recently identified to be phosporylated on four arginine residues (7). As the deletion of McsB seems to stabilize the GFP-GudB^{CR} fusion protein it will be interesting to study whether the phosphorylation on arginine residues is a prerequisite for efficient degradation of GudB^{CR}. The results may help to gain further insights into the physiological function of arginine phosphorylation in *B. subtilis*.

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GRP027

Exploring the lantibiotic autoinduction by use of subtilin/nisin hybrid-peptides

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Subtilin and nisin are representatives of the lantibiotics [1]. These are peptide derived antibiotics, produced by and active against Gram-positive bacteria. The ribosomally synthesized precursor peptides are posttranslationally modified, thereby resulting in the dehydration of serine and threonine residues and the development of thioether bridges formed by meso-lanthionine and 3-methyllanthionine. Subtilin and nisin differ in 14 amino acids but share a highly similar lanthionine ring structure consisting of five lanthionine rings. Despite their structural similarity, they are highly specific for their respective autoinduction system. The producer strains *Bacillus subtilis* and *Lactococcus lactis* sense extracellular subtilin respectively nisin via a two component system consisting of a histidine kinase and a response regulator. The histidine kinase is autoinduced in a quorum sensing manner and phosphorylates the response regulator, which in turn induces the expression of the lantibiotic structural gene, the genes of the lantibiotic biosynthesis machinery and the self immunity genes [2, 3].
So far the interaction between subtilin or nisin with their corresponding histidine kinase remains unclear. To identify the specific binding motif of subtilin and nisin an expression system has to be established which is capable to generate both, subtilin and nisin and furthermore subtilin/nisin hybrid-peptides. Employing a β-galactosidase based reporter system for lantibiotic autoinduction these hybrid molecules will be used to elucidate the specifity of binding between the lantibiotic and its corresponding histidine kinase by virtue of their specific autoinduction.

Additionally, these molecules will be used to analyse and optimize the lantibiotic properties with respect to the activity, stability and solubility.

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GRP028

Exploring endophytic colonization mechanism of nitrogen-fixing bacterium *Azoarcus* sp. BH72 in rice

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Rice is one of the world's staple foods, but in recent years its production growth fell behind the growth of the comsumer population. In agriculture increasing nitrogen-uptake is one important way to improve rice growth. However, the commonly used industrial fertilizers are environmentally problematic. As an alternative, biologically fixed nitrogen can be the future for its reduced production cost and less environmental risk. *Azoarcus* sp. BH72 is a nitrogen-fixing endophyte isolated from Kallar grass. It can also colonize the rice roots gnotobiotically and promote rice growth, as one of the many rice endophytes. However, the mechanism of establishing this relationship is unknown, hence here *Azoarcus* is taken as a model to study the interaction. In our study, the rice roots gene expression profiles in response to *Azoarcus* colonization were analyzed with microarrays (Agilent). In addition, the transcriptome of rootes infected with the rice blast pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) was analyzed to compare endophyte and pathogen-induced rice immune responses. First, the results show evidences supporting the mutual-beneficial lifestyle. Rice might be the carbon source (ethanol and malate) supplier of *Azoarcus*: Compared with non-infected rice roots, *Azoarcus*-colonized ones showed up-regulated expression level of genes coding for alcohol dehydrogenase that catalyzes ethanol production. Furthermore, a gene coding for malate tonoplast transporter was also down-regulated, which might reduce the vacuole storage of malate and increase the exuded ones feeding *Azoarcus*. On the other hand, *Azoarcus* might supply rice with nitrogen in forms of ammonium, amino acid and peptide, indicated by an enhanced gene expression of their corresponding transporter and an aminotransferase in the colonized rice roots. 17 leucine-rich repeat receptor-like kinases were also found up-regulated as *Azoarcus*-receptor candidates. Especially three of them were moderately similar to the root nodule symbiosis-related receptor-like kinase. A salicylic acid and a jasmonic acid-induced immune response were found in both *Azoarcus* and *Xoo*-colonized roots.

GRP029

Lactose- and galactose- mediated gene regulation in *Streptococcus pneumoniae* and the repressor function of the novel DeoR-type regulator LacR

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Streptococcus pneumoniae has the ability to colonize the human nasopharynx and causes serious infections like otitis media, pneumonia, meningitis and bacteremia. Transcriptomic analysis in the presence of lactose compared to glucose and galactose compared to glucose revealed elevated expression of two gene clusters in the *S. pneumoniae* strain D39, which encode the tagatose pathway (*lacABCD*) and lactose specific-phosphotransferase system (PTS) enzyme II permeases (*lacEF*) genes. Genes encoding a predicted phospho-β-galactosidase (LacG), a DeoR family transcriptional regulator (LacR), and a transcriptional antiterminator (LacT) are also present in these clusters and are highly upregulated in the presence of lactose and galactose. Deletion of *lacR* revealed high expression of genes (*lacABCD*) involved in tagatose pathway, even in the presence of glucose. This suggests that LacR functions as a repressor of the genes (*lacABCD*) involved in tagatose pathway in the absence of lactose/galactose. This is further confirmed by β-galactosidase assays with P*lacA-lacZ* in the presence of lactose, galactose and glucose as sole carbon source in the medium. Moreover, deletion of *lacR* has no effect on the expression of lactose specific PTS genes (*lacEF*). This suggests a putative role of another transcriptional regulator in regulation of lactose-specific PTS ,which might be LacT. Deletion of *lacEFG* slightly affects the normal growth of D39 strain on lactose. This indicates the existence of another lactose specific PTS in D39. We are currently engaged in elucidating the role of LacT and CcpA in galactose and lactose transport in *S. pneumoniae*.

GRP030

Raa4 promotes splicing of a chloroplast group II intron in *Chlamydomonas reinhardtii*

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The expression of the chloroplast genome is highly dependent on a large number of nucleus-encoded factors which are believed to promote the maturation of chloroplast precursor RNAs and could be part of a putative chloroplast spliceosome [1]. In the green alga *Chlamydomonas reinhardtii* the chloroplast *psaA* gene is split into three independently transcribed exons, which are flanked by consensus sequences of group II introns [2]. At least 14 nucleus-encoded factors and a small chloroplast-encoded RNA (*tscA*) are required for the maturation of the *psaA* mRNA. We are interested in identification and functional characterization of components from the putative chloroplast spliceosome. In this regard by using forward genetic approach, we have generated *trans*-splicing mutants and rescued one of these mutants by genomic complementation [3]. The affected gene is called *RAA4*, for RNA maturation of *psaA* 4. Raa4 does not share significant sequence similarities with any other proteins except for a small domain present in aminoacyl t-RNA synthetases. Here, we present the functional analysis of this protein by electrophoretic mobility shift assays and localisation studies. The yeast two-hybrid system was used for the detection of putative Raa4 interaction partners.

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GRP031

Identification of a chloroplast ribonucleoprotein complex containing trans-splicing factors, intron RNA and novel components

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Chlamydomonas reinhardtii is widely used for analysis of nucleus-encoded factors that are thought to promote the maturation of chloroplast precursor RNAs. To elucidate the function and composition of ribonucleoprotein complexes that are presumably part of a transcript specific chloroplast spliceosome, we are studying the expression of the chloroplast encoded psaA gene [1]. The psaA gene is separated into three exons, which are widely distributed over the plastom and flanked by consensus sequences typical for group II introns. The exons are transcribed individually and the major transcript is then assembled in trans. Here, we present a novel trans-splicing mutant, which is affected in splicing of the first psaA intron. Genomic complementation led to the identification of the mutant gene encoding Raa4, a protein of 112.4 kDa, which shares no strong sequence identity with other known proteins [2]. The chloroplast localization of Raa4 was confirmed by confocal fluorescence microscopy, using a GFP-tagged fusion protein. RNA binding-studies showed that Raa4 binds specifically to domains D2 and D3, but not to other conserved domains of the tripartite group II intron. In addition, we used a combined experimental approach including yeast-two hybrid screening, tandem affinity purification (TAP) and mass spectrometry to identify putative interaction partners of Raa4.

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GRP032

Purification of Raa4 complexes using a codon-optimized TAP tag: Novel protein-protein interactions between chloroplast splicing factors

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The chloroplast biogenesis in Chlamydomonas reinhardtii is dependent on various nucleus-encoded trans-acting factors. In case of the psaA gene, which encodes one of the P700 chlorophyll a/ b binding proteins of photosystem I, three independent transcribed exons are spliced in trans [1].

The factor Raa4 is involved in splicing exon 1/2 [2]. It was used as bait in tandem affinity purification (TAP) to identify new components of a putative chloroplast spliceosome. Therefore a new codon optimized TAP tag variant was generated and fused to the Raa4 bait protein. After TAP under native conditions, mass spectrometric analysis of the eluate revealed Raa4 and its specific interaction partners. Wild type and RbcS1-TAP control purifications were used to exclude false-positive interactions. Comparison of TAP using cultures grown under different environmental conditions (light, dark, anaerobiosis) led to the identification of 23 putative Raa4 interaction partners identified in at least three-out-of-four (light) or two-out-of-three (dark, anaerobiosis) replicates. Among those were the previously described trans-splicing factors Raa1, Rat2 and Raa3 [3, 4, 5]. Furthermore, qRT-PCR with RNA purified from Raa4-TAP protein eluates was performed indicating a significant enrichment of spliceosomal RNA compared to wild type TAP eluates.

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GRP033

In vitro characterization of the YycFGHI regulatory system of *Staphylococcus aureus*

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YycFG (WalRK/VicRK) represents the only essential two-component system in the nosocomial gram positive pathogen *Staphylococcus aureus*. While knowledge on localization and regulatory activity of this essential system has steadily increased since its discovery in 1998 [1][2][3], less is known about the properties of the kinase YycG. To address this question, the full-length recombinant proteins of the YycFGHI operon have been expressed and tested under *in vitro* conditions, using Triton X-100 as a membrane mimicking surfactant or phospholipid-liposomes, respectively. Suitable conditions for autophosphorylation of the full-length YycG kinase and subsequent phosphoric group transfer to YycF could be identified. High alkali salt concentrations (particularly KCl) and low temperatures were necessary to stimulate YycG activity, indicating that the kinase might respond *in vivo* to membrane fluidity/stiffness via its TM domains [4]. Previous reports had suggested that the membrane-tethered auxiliary proteins YycH and YycI act as regulators in a negative manner on YycG [5][6][7]. Furthermore, this system has repeatedly been reported to be involved in the intermediate resistance of *S. aureus* to vancomycin and daptomycin. Here, we investigate the influence of different parameters on the autophosphorylation activity of the kinase and phosphotransfer to the response regulator protein.

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GRP034

Transcriptional response of *Streptococcus pneumoniae* to varying sources of iron and the regulatory mechanism of the iron uptake system PiuBCDA

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For several decades the battle for iron between bacterial human pathogens and their hosts has been studied, showing that the pathogen exploits an extensive arsenal of factors to obtain sufficient iron. The host, on the other side, attempts to restrict availability of free iron in its tissues. Streptococci, comprising an important group of human pathogens, harness diverse iron acquisition systems to be fully virulent. However, the role of iron on the global gene regulation of the Gram-positive human pathogen *Streptococcus pneumoniae* has not been explored yet. The genome of *S. pneumoniae* D39 encodes three operons for iron transport systems. Regulation of these operons has not been studied till to date. In this study, we explore the transcriptional responses of *S. pneumoniae* D39 to different sources of iron (Fe^{2+}, Fe^{3+} and Heme). The transcriptomic data on these strains grown with different iron source additions revealed that out of the three known iron transport operons, only *piuBCDA* was involved in iron (Fe^{2+} and Fe^{3+}) uptake. Moreover, the role of two transcriptional regulators, i.e. RitR (Repressor of iron transport) and CodY (global nutritional regulator) in the regulation of the *piuBCDA* operon was studied. Here we show that RitR represses the expression of the *piuBCDA* operon in the presence of iron. This is further confirmed by transcriptome studies with a *ritR* mutant in the presence of iron. Notably, CodY seems to act as an activator for this operon. A mutation in the CodY binding site present in the P*piuB* led to full repression of the *piuBCDA* operon by RitR. In conclusion, we have explored the complex regulation of the important *piuBCDA* operon by RitR and CodY, and assessed the global effect of iron on gene expression of *S. pneumonia*, including genes known to be important for virulence.

GRP035

Iron and zinc-dependent regulation in the strict anaerobe Clostridium acetobutylicum

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In the natural environments, bacteria are continuously challenged with either insufficient or elevated and even toxic amounts of metals. Therefore, they had to establish a tight control on the intracellular metal content in order to meet the metabolic needs of the cell. Classical metal-dependent response in bacteria is carried out by the Fur (ferric uptake regulator) family of regulators. These proteins function as dimeric transcriptional repressors that bind to palindromic sequences in the promoters of the target genes. This family includes members like Fur and Zur, which sense two distinct divalent metals certainly of great importance for almost all microorganisms- iron and zinc, respectively.
The genome of the solvent-producing, endospore-forming strict anaerobe Clostridium acetobutylicum revealed two genes encoding a putative ferric uptake regulator (Fur) and a putative zinc uptake regulator (Zur). We inactivated fur and zur genes through insertional mutagenesis using the Clostron system and both mutants were physiologically characterized. To gain further insights into the role of the Fur and Zur proteins and the mechanisms for establishment of metal balance in C. acetobutylicum, we characterized the gene expression profile of the mutants and the iron- respectively zinc-limitation stimulon of the parental strain. Our results demonstrated that C. acetobutylicum senses and responds to availability of iron and zinc on multiple levels using a sophisticated system and the Fur family of proteins plays an important role in this process.

GRP036

Dissection of the Regulatory Networks Controlled of the ECF Sigma Factors SigE and SigH in *Corynebacterium glutamicum*

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Expression of genes in *C.glutamicum*, a member of the *Actinomycetales* used mainly for the industrial production of amino acids, is regulated by seven different sigma factors, five of them belonging to the "extra-cytoplasmic function" (ECF) family, including SigH and SigE, which are cytoplasmic-sensing ECF factors [1]. Sigma factors of the ECF family are widespread, non-essential and control regulatory networks involved in adaptation to environmental stresses in bacteria. To dissect these regulatory networks is a challenging task.
The SigH-dependent transcription in response to thiol-oxidative stress is controlled by an anti-sigma factor RshA in *C. glutamicum* [2], similar to the related species *Mycobacterium tuberculosis* and *Streptomyces coelicolor*, where the orthologous RsrA controls the SigH ortholog SigR [3]. In these bacteria, SigE (ECF group 14) and its orthologs, although not sharing a high degree of sequence similarity to SigH orthologs (ECF 12) [1], have a partly redundant action and address similar promoter sequences [4]. The aim of our study was to analyze the roles of SigH and SigE and their overlapping regulons in *C. glutamicum* in depth.
For this purpose we developed a method to untangle hierarchies and interactions in complex transcriptional regulatory networks by a combination of RNA-seq (deep sequencing of transcriptomes) of sigma-factor knock-out mutants (*in vivo* top-down) and run-off transcription approaches (*in vitro* bottom-up). The *in vitro* method is a variation of the *ROMA* (*Run-Off transcription analysed by Micro-Arrays*) approach [5], and we call it *ROSE* (*Run-Off transcription analysed by SEquencing*). The

results of this combined approach for dissecting the regulatory networks controlled by SigH and SigE in *C. glutamicum* will be presented.
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GMV001

The MIMAS project and beyond: A collaborative effort for gaining detailed insights into carbohydrate-degrading marine bacteria with a focus on algal blooms in the German bight

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Marine algae contribute about half of the global biological carbon fixation, and thereby provide the basis of the marine food web of micro-, meio- and macrofauna from protists to fishes, as well as the heterotrophic bacterioplankton. We studied the response of the bacterioplankton to spring phytoplankton blooms in the German Bight and observed swift responses of *Flavobacteria* and *Gammaproteobacteria* with different niche specializations in macromolecule degradation, in particular carbohydrates. Initially, we used a combination of biodiversity assessments, metagenomics, metaproteomics, and environmental parameter measurements for our studies. Lately we complemented this repertoire by targeted cultivation efforts in conjunction with draft genome sequencing and proteomics, as well as environmental carbohydrate-detection. Initial results demonstrate that this combined data integration approach bears the potential to uncover the niches of environmentally relevant carbohydrate-degrading bacteria in an as yet unseen level of detail, and thus to substantially deepen our understanding of an as yet under-researched aspect of the global carbon cycle.

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GMV002

An arsenal of toxins in the genome of symbionts from deep-sea hydrothermal vent mussels

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Bathymodiolus mussels are prominent members of deep-sea hydrothermal vent and cold seep communities worldwide. The key to their success is their association with symbiotic chemosynthetic bacteria that provide them with nutrition. The mussel symbionts are horizontally transmitted, and each new host generation must be infected by free-living symbionts. However, nothing is known about the mechanisms the symbionts use to invade and survive within host cells. We obtained a 2.3 Mb draft genome of the chemoautotrophic symbiont of *Bathymodiolus* sp. from a hydrothermal vent in the Atlantic. We compared the *Bathymodiolus* symbiont genome to those of its closest free-living relatives, the widespread pelagic sulfur oxidizers SUP05, and its closest symbiotic relatives, the vertically transmitted obligate symbionts of vesicomyid clams. Our comparative genomic analyses revealed that the *Bathymodiolus* symbiont has undergone massive rearrangements, and that as much as 38% of its genes may be of foreign origin, in contrast to 25% in the clam symbionts and 29% in SUP05. Intriguingly, many of the genes unique to the *Bathymodiolus* symbiont were homologs of virulence genes. We discovered a diverse array of toxins with highest similarities to insecticidal toxins encoded by the *Xenorhabdus* and *Photorhabdus* symbionts of nematodes, and toxins of pathogens such as *Yersinia* and *Vibrio*. These included at least 9 RTX toxins, 29 YD repeat toxins, and up to 20 MARTX toxins. Characterized members of these toxin classes can cause pore formation in infected host cells, cytoskeleton remodeling, apoptosis inhibition, and macrophage inactivation. The phylogeny of the *Bathymodiolus* symbiont toxin genes, their codon usage patterns, and proximity to genes and regions known to play a role in DNA transfer suggest that they were horizontally acquired. We found many of these toxins in a published transcriptome of symbiont-hosting *Bathymodiolus* gill tissues, and in our preliminary proteomic analyses. It is therefore highly likely that the chemoautotrophic symbionts are actively expressing these toxins in their hosts. We found a similarly high number of toxins in the chemoautotrophic symbiont genome from another *Bathymodiolus* species, but not in the genomes of chemosynthetic symbionts from other hosts such as tubeworms, shrimps or clams. The *Bathymodiolus* symbiont genome therefore encodes a unique arsenal of toxins, unprecedented in beneficial symbionts. We hypothesize that the symbionts may have 'tamed' these toxins to use them in beneficial interactions with its host.

GMV003

Unraveling the unknowns in the metagenomic protein universe using Graphical Models

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Metagenomic environmental surveys, like the Global Ocean Survey (GOS), generated a huge amount of genetic data and allow performing more holistic approaches to study marine ecosystems. Moreover, metagenomics proofed being valuable in discovering missing links in marine biological processes. Besides expanding our limited view on the diversity of the known protein universe, metagenomics also revealed a large number of genes of *unknown* functions. These can be further classified into I) *known unknowns* like the domains of unknown function (DUF) and II) *unknown unknowns*, putative coding sequences without any hint of potential function. We will present a novel approach to extract valuable information from the co-occurrence of individual protein domains involved in biological processes using Graphical Models. Using an integrative approach, we combine the knowledge of the known protein domain families and 16S ribosomal DNA with the *unknown unknowns* to explore the GOS metagenome. As a result, we were able to reveal new associations in biological processes within known protein families and between known protein families and *unknowns*.

GMV004

Expression profile analysis of oxygen response in the nitrogen-fixing endophyte *Azoarcus* sp. bh72 by genome-wide dna microarray

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Low oxygen tensions are often encountered in flooded soils by root-associated *Azoarcus* sp. BH72 which fixes nitrogen only under microaerobiosis. In this study, genome wide oligonucleotide microarrays were used to analyze the global transcription profile of strain BH72 under aerobic and microaerobic conditions, with ammonia as sole nitrogen source. Approximately 8.7% (350 of 3989) genes were significantly modulated more than 1.8-fold (P-value≤0.05) in response to oxygen. Out of 235 genes specifically up-regulated under microaerobiosis (0.3% oxygen), several hypothetical proteins as well as genes involved in energy metabolism were

overrepresented with specific up-regulation of genes for *cbb3*-type terminal oxidases and NADH dehydrogenases. Indeed a transcriptional *ccoN::gusA* fusion strain of *Azoarcus* sp. BH72 encoding for *cbb3*-type terminal oxidase exhibited 2.4 fold elevated expression only under microaerobiosis. In agreement with expression studies, a *ccoN* mutant of strain BH72 was impeded in growth under microaerobic N_2-fixing conditions. Dithionite-reduced minus air-oxidized difference spectra of membrane preparations typical of cbb_3 oxidases in the wild type changed significantly in the mutant, showing an apparent shift to *b*-type cytochromes. Aerobic condition (21% O_2) on the other hand induced expression of several genes mainly involved in oxidative stress protection. Microaerobic response was sufficient enough to induce several genes related to N_2 fixation or denitrification independent of ammonia or nitrate, respectively. It even induced several genes putatively involved in secretion, plant colonization and rhizosphere competence. Furthermore, out of 176 genome-wide predicted conserved sequences for FNR (global anaerobic regulator) binding, 33 overlapped in the upstream region of target genes which got up-regulated under microaerobiosis too including that of *ccoN*: the first gene of the *cbb3*-type oxidase gene cluster. Accordingly expression of 11 transcriptional regulators was specifically modulated under microaerobiosis. Expression of several candidates was further verified by qRT-PCR. The outcome of this study provided a better insight about the establishment in a microaerobic environment, putatively prevailing in the niche of this endophyte, rice roots.

GMV005

Metatranscriptomic analysis of rice straw degradation by paddy soil microbial communities

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Rice straw is a complex biopolymer composed of cellulose, hemicelluloses, lignin, and water-soluble polysaccharides. Its incorporation into paddy soil is a common agricultural practice. Organic carbon from rice straw is primarily released by anaerobic degradation, resulting in increased rates of CH_4 and CO_2 production. Here, we analyzed the temporal succession and global gene expression of microbial communities involved in the degradation of rice straw. The expression of glycosyl hydrolases, key enzymes in the degradation of complex polysaccharides, was of particular interest.
Paddy soil slurries amended with rice straw were incubated under anoxic conditions for 4 weeks at 28°C. Samples for molecular analysis were taken at 0, 1, 7, and 28 days. Analysis of both rRNA and mRNA was performed by 454-pyrosequencing.
Rice straw amendment induced major changes in community composition already after 1 day of incubation. These were defined by a decline of *Geobacteraceae* that were prevalent in the non-incubated paddy soil, and a strong increase in the abundance of *Clostridiales*, including members of the *Clostridiaceae*, *Lachnospiraceae*, *Veillonellaceae* and *Ruminococcaceae*. Comparative mRNA-tag analysis showed that transcripts related to anaerobic respiration and methanogenesis were overrepresented after 7-day incubation. Transcripts encoding glycosyl hydrolases, carbohydrate transporters, and receptor activity, and, in general, those involved in cellulose, chitin and hemicellulose metabolism were more prevalent after 28-day incubation. The relative proportion of mRNA tags annotated as glycosyl hydrolases increased from 2 to 6%. This increase coincided with a broader spectrum of substrates. Similar but slightly time-delayed changes in the rRNA and mRNA patterns were observed between rice straw fraction and paddy soil.
In summary, members of the *Clostridiales* were the key players in the degradation of rice straw, thereby reflecting their metabolic versatility. The increase in the relative abundance and diversity of transcripts encoding glycosyl hydrolases underlines both major shifts in community function and the importance of this enzyme family for the degradation of plant polymers.

GMV006

Genome based methods for the exploration of natural products from marine fungi for the treatment of cancer

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Despite marine fungi are a potent group of secondary metabolite producers, they are not well characterised and underutilised in terms biotechnological application. Here, we demonstrate the sustainable exploitation of marine natural resources providing appropriate culture conditions for the group of marine fungi, thus enabling efficient production of marine natural products in the laboratory and also in large scale cultures, avoiding harm to the natural environment. In the focus are new anti-cancer compounds. Beside isolation of new fungal strains from unique marine habitats, the molecular development of effective producer strains is in the focus. Genomes of selected candidate strains originating from our unique strain collection of marine fungi are characterised with respect to secondary metabolite production. This knowledge is use to optimise production using molecular methods.
This approach is an outcome of the project "MARINE FUNGI" within the KBBE framework of EU's FP7. We develop a process concept for these compounds providing the technological basis for a sustainable use of marine microbial products as result of “Blue Biotech”.
www.marinefungi.eu

GMV007

Sequencing and annotation of the genome of the mercaptosuccinate utilizing proteobacterium *Variovorax paradoxus* strain B4

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Strains of the genus *Variovorax* are known for their high metabolic capabilities. Among other things, they are able to catabolize a wide range of organic sulfur compounds such as sulfolane, taurine, mercaptosuccinate or aromatic sulfonates of which some are found in higher concentrations in polluted environments [1, 2, 3]. We sequenced and annotated the genome of *Variovorax paradoxus* strain B4 to elucidate the utilization of mercaptosuccinate and other sulfur compounds in this strain [1]. Mercaptosuccinate represents a potential novel precursor substrate for the production of polythioesters, and investigating the metabolism of this substance could be useful for the optimization of future production strains. Another aspect is the identification of additional interesting features of strain B4 also in comparison to *V. paradoxus* strains S110 and EPS. The genome of strain B4 comprises a total of 7,015,925 bp, and it consists of two separate chromosomes: chromosome 1 containing 5,795,261 bp and chromosome 2 containing 1,220,664 bp with average GC contents of 67.7% and 67.5%, respectively. A number of 6,755 putative protein coding genes were predicted of which many are putatively involved in the utilization of organic sulfur compounds. Comparative genomics with the closely related *V. paradoxus* strains S110 [4] and EPS pointed out that this genus exhibits a remarkably high capability of utilizing organic sulfur compounds as well as xenobiotics. These results were confirmed by growth experiments using the three strains and different sulfur-containing substrates as source of carbon and energy or sulfur.

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GMV008

A computational perspective on micro-bial secondary metabolite biosynthesis

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Microbial secondary metabolism is a rich source of bioactive compounds with potential pharmaceutical applications[1]. In the past decade, computational methods have become more and more important to exploit this potential.
In order to devise effective strategies to make full use of the accelerated rate of genome sequencing, we recently constructed antiSMASH (http://antismash.secondarymetabolites.org), the first comprehensive pipeline capable of identifying biosynthetic loci covering the whole range of known secondary metabolite compound classes[2]. antiSMASH automatically integrates chemical structure predictions, domain analysis of modular enzymes, alignments between homologous gene clusters and phylogenetic analysis.
Combining antiSMASH with a generic algorithm that identifies genomic regions involved in small molecule biosynthesis, we have now performed a global quantitative and comparative analysis of biosynthetic gene clusters in all microbes (Cimermancic, Medema et al., in preparation). The results offer new leads towards the identification of radically novel classes of molecules.
We have devised strategies to implement identified gene clusters in high-throughput synthetic biology methodologies for activity screening and industry-scale production[4-6]. To support these efforts, we are developing a number of computational methods[7], such as comparative genome-scale metabolic modelling of secondary metabolite production to inform optimal host selection[8] and automated homology searches to identify new operon and gene cluster parts in biological sequence databases[9].
When combined with solid experiments, these bioinformatic approaches have great promise to advance the study of microbial biosynthesis and inform optimal experimental design.

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GMP001

Phylogenetic analysis of a Lake Constance Bacterio-plankton metagenome

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Lake Constance is a large, monomictic pre-Alpine lake and represents one of the best studied lakes in Europe. Particularly the annual succession of the phyto- and zooplankton in Lake Constance has been examined in great detail over more than 30 years. However, the diversity of the pelagic microbial community in Lake Constance remained largely unexplored. A bacterioplankton community composition analysis was performed using a metagenome dataset derived from filtrates of Lake Constance epilimnia. Overall, the observed bacterioplankton community represented a typical oligotrophic freshwater lake community, in both, composition and abundance. The average distribution was dominated by the phyla *Proteobacteria* (30 - 46 %) and *Actinobacteria* (22 - 30 %), in addition to *Cyanobacteria* (7 - 18 %), *Bacteroidetes* (6 - 11 %), and *Verrucomicrobia* (1 - 13 %). Analysis of their distribution within different size classes revealed that the represented bacterial lineages were previously recognized to be found exclusively in freshwater lakes.
A protocol for a routine sampling of bacterioplankton, DNA extraction, and community analysis has been established and was used to collect samples over a full seasonal succession. Currently, major changes in the community structure are identified by denaturing gradient gel electrophoresis (DGGE).

GMP002

Analyzing microbial diversity using the metagenomics module of bionumerics®

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The advent of low cost next-generation sequencing methods is having an effect on the study of the microbial world, on the field of metagenomics and the study of uncultured bacterial diversity. Direct investigation of the vast majority of bacteria, irrespective of their cultivability and taxonomic identities, has challenged the concept of species diversity and has led to various new insights into the composition and functionalities of microbial communities. To analyze and assess the microbial diversity, most studies use open source packages or a variety of scripts.
We developed a tool in the BioNumerics® (BN) software for the analysis, quantification, visualization and comparison of microbial communities starting from sequence reads, independent of the sequencing platform used. Within the graphical user interface, the concept of a metagenomics analysis workflow is used to which actions can be added or deleted to their own interest.
BN makes use of the **mothur**[1] project, which filled in the needs of the microbial ecology community by incorporating the functionality of numerous applications into one command line application. BN uses the flexibility of the algorithms implemented in mothur and elaborates on these results by creating an interactive reporting service including a geographical visualization tool and various chart tools for the interpretation and manipulation of the results. The integrated follow-up analysis includes data mining and statistics. In this study, we illustrate this new tool with publicly available genomic data sets.
BN offers one environment to start from the raw read sequences, perform trimming, chimera removal and sequence clustering to end up with visualization of the OTU abundances or the evaluation of the diversities using a plethora of indices. Performing each analysis step is facilitated by an intuitive user interface and visual feedback flexible reporting tools. The integration of the metagenomics functionality enables the combination of formerly used environmental analyses with the newly obtained metagenomics results to allow comparison between the different methods.

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GMP003

European foulbrood of honeybees: Genomic approaches.

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Honeybees are the most important pollinators of crop monocultures worldwide (2). European foulbrood (EFB) is a bacterial disease caused by *Melissococcus plutonius*. EFB occurs worldwide with increasing appearance in some areas such as Great Britian (3) and Switzerland. EFB affects mainly unsealed brood and honey bee larvae are usually killed at an age of 4-5 days (1). In general, low numbers of bacterial cells are sufficient to cause infection. *Paenibacillus alvei*, *Brevibacillus laterosporus*, *Enterococcus faecalis* and "*Achromobacter eurydice*" occur as secondary invaders during outbreaks of EFB.
Sequencing of the *M. plutonius*, *P. alvei* and *B. laterosporus* genomes were done by using a combination of 454-pyrosequencing and Illumina techniques. The obtained sequences were assembled, contigs sorted, and remaining gaps closed. The genome size of *M. plutonius* is approximately 2.1 Mb with an overall G+C content of 31 mol%, while *P. alvei* DSM 29 and *B. laterosporus* LMG 15441 comprise genome sizes of approximately 6.8 Mb (45.9 mol% GC content) and 5.1 Mb (41.1 mol% GC content), respectively. Genome sequence analysis of secondary invaders revealed large potential to produce polyketides, nonribosomal peptides and toxins. *M. plutonius* harbors a mannose transporter phosphotransferase system, which is known for its broad substrate specificity, and pectin-degrading enzymes.

These results indicated pollen perforation *M. plutonius,* which results in release of nutrient-rich pollen content. Additionally, *M. plutonius* harbors genes coding for S-Layer components and enhancin, which might contribute to its invasive capacity.

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GMP004

Genome analysis of *Advenella mimigardefordensis* strain DPN7^T and elucidation of the catabolic pathway of 3,3´-dithiodipropionic acid

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A. mimigardefordensis strain DPN7^T is an interesting *β*-proteobacterium due to its extraordinary capacity to use 3,3´-dithiodipropionic acid (DTDP) as sole source of carbon and energy for growth [1]. DTDP is a non-toxic precursor substrate for microbially synthesized polythioesters (PTE), which are non-degradable biopolymers and thus represent an interesting bioplastic [2]. Metabolic engineering for optimization of PTE production requires the entire understanding of DTDP conversion. Consequently, the genome of *A. mimigardefordensis* strain DPN7^T was sequenced and annotated, and is now available for further studies: The complete genome of *A. mimigardefordensis* strain DPN7^T comprises 4,764,126 bp and is distributed on two replicons: One circular chromosome consisting of 4,740,516 bp and a plasmid of 23,610 bp, composed of 4,112 and 24 predicted open reading frames, respectively. Putative functions were assigned to 3692 (89.26%) of all protein-coding sequences. The genome harbors 7 pseudogenes, 39 tRNA genes and two copies of rRNA operons. The average GC-content amounts to 54.22%, which facilitates molecular biological procedures. All metabolic genes participating in the DTDP catabolism were now unraveled: DTDP is most probably transported into the cell via one specific tripartite tricarboxylate transport system and is afterwards cleaved into two molecules of 3-mercaptopropionic acid (3MP) by the disulfide-reductase LpdA. 3MP is then oxygenated by the 3MP-dioxygenase (Mdo) yielding 3-sulfinopropionic acid, which is activated to the corresponding CoA thioester by the CoA ligase SucCD. The next step is the abstraction of sulfite from 3-sulfinopropionyl-CoA by a novel reaction of the acyl-CoA dehydrogenase Acd, and subsequently propionyl-CoA enters the central metabolism via the methycitric acid cycle. Regulation of this pathway is presumably realized by a transcriptional regulator of the XRE (xenobiotic response element) family, which is located on the chromosome in the vicinity of *acd* and *mdo*.

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GMP005

A genomic approach to the cryptic secondary metabolome of the anaerobic world

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In terms of natural products, one group of highly neglected bacteria are those that grow without oxygen: the anaerobes. A major obstacle to the investigation of natural products in anaerobes comes from the long held belief amongst secondary metabolite researchers that these bacteria are incapable of producing secondary metabolites.[1] The discovery of closthioamide[2], the first antibiotic from the obligate anaerobe *Clostridium cellulolyticum*, refuted this.
A total of 211 completed and published genomes from anaerobic bacteria were analysed by bioinformatic techniques for the presence of secondary metabolite gene clusters, in particular those belonging to polyketide synthases (PKS) and non-ribosomal peptide synthetases (NRPS).[3] We investigated the distribution of these clusters according to bacterial phylogeny and, if known, correlated these to the metabolites they synthesize. It could be shown that, contrary to popular belief, many anaerobes have the potential to produce polyketides and/or nonribosomal peptides. At present, both Firmicutes of the genus *Clostridium* and anaerobic δ-proteobacteria appear to be the most promising sources for many potential new molecules waiting to be identified from the "anaerob-ome".

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GMP006

Ocean Sampling Day: A community effort towards a better understanding of our oceans

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Recent developments in sequencing technology make routine sequencing of whole microbial communities from the environments a widely used and affordable routine task. Consequently, large scale sampling and sequencing efforts such as the Global Ocean Sampling (GOS) campaign, the Malaspina cruise and the Tara Oceans expeditions are now exploring marine ecosystems in space and time. In contrast to such circumnavigations, no orchestrated site-based and fixed in time sampling effort has been initiated so far. The EU funded FP 7 project Micro B3 (Biodiversity, Bioinformatics, Biotechnology, http://www.microb3.eu) is now taking action to implement the Ocean Sampling Day (OSD, http://www.oceansamplingday.org). It will be conducted on the summer solstice (June 21st) in the year 2014. The OSD will involve all Micro B3 partners with study sites and any interested lab across Europe and beyond. These cumulative samples, fixed in time and space supplemented with a broad set of geo-referenced environmental parameters, will contribute to determine a baseline of marine biodiversity and functions on the molecular level. To ensure maximum usefulness of these samples, sampling and data analysis will be done across all sites using agreed upon best practices developed within Micro B3. Herewith, a high level of consistency between data points across Europe and beyond is ensured. In particular all sites will be expected to comply to the minimum information checklists of the http://www.gensc.org for describing 'Omics samples. All data will be made available to the public. We expect that these data will provide a reference data set for generations of experiments to come. It should also function as starting point for site-based monitoring of microbial communities as proposed by the Genomic Observatories initiative.

Reference:
Davies, N., Field, D., Glöckner, F.O. & The Genomic Observatories, N. Sequencing data: A genomic network to monitor Earth. Nature 481, 145-145 (2012).

GMP007

Automated, Non-Hybrid *De Novo* Genome Assemblies and Epigenomes of Bacterial Pathogens

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Understanding the genetic basis of infectious diseases is critical to enacting effective treatments, and several large-scale sequencing initiatives are underway to collect this information[1]. Sequencing bacterial samples is typically performed by mapping sequence reads against genomes of known reference strains. While such resequencing informs on the spectrum of single nucleotide differences relative to the chosen reference, it can miss numerous other forms of variation known to influence pathogenicity: structural variations (duplications, inversions), acquisition of mobile elements (phages, plasmids), homonucleotide length variation causing phase variation, and epigenetic marks (methylation, phosphorothioation) that influence gene expression to switch bacteria from non-pathogenic to pathogenic states[2]. Therefore, sequencing methods which provide complete, *de novo* genome assemblies and epigenomes are necessary to fully characterize infectious disease agents in an unbiased, hypothesis-free manner.
Hybrid assembly methods have been described that combine long sequence reads from SMRT® DNA sequencing with short reads (SMRT CCS or second-generation reads), wherein the short reads are used to error-correct the long reads which are then used for assembly. We have developed a new paradigm for microbial *de novo* assemblies in which long SMRT sequencing

reads (average readlengths >5,000 bases) are used exclusively to close the genome through a hierarchical genome assembly process, thereby obviating the need for a second sample preparation, sequencing run and data set. We have applied this method to achieve closed *de novo* genomes with accuracies exceeding QV50 (>99.999%) to numerous disease outbreak samples, including *E. coli, Salmonella, Campylobacter, Listeria, Neisseria,* and *H. pylori*. The kinetic information from the same SMRT sequencing reads is utilized to determine epigenomes. Approximately 70% of all methyltransferase specificities we have determined to date represent previously unknown bacterial epigenetic signatures. The process has been automated and requires less than 16 hours from an unknown DNA sample to its complete *de novo* genome and epigenome.

[1]e.g., the 100K Foodborne Pathogen Genome Project (www.100kgenome.vetmed.ucdavis.edu/)
[2]Srikhanta et al. (2010) *Nat Rev Microbiol* 8: 196-206.

GMP009

Metagenomic analysis using long 16S amplicons and the Roche 454 GS FLX+ platform

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Objectives: Metagenomic analyses using 16S rRNA or other amplicons are mostly limited to short reads of 100-500bp due to the maximal read length of the applied sequencing platforms. The release of the 454 GS FLX+ sequencing platform enables the combination of reads up to 1kb with high throughput sequencing, but is still limited to shotgun adaptor libraries. In this study we assessed and optimized the usability of this platform in sequencing long amplicon libraries for metagenomic analyses.
Methods: For this purpose we used a mock community with species derived from various habitats (e.g. human associated, soil, aquatic) for generating libraries comprising long amplicons. We used Lib-L fusion primers which amplified products longer than 780bp and spanning multiple 16S variable regions (e.g. V3 to V6)
Results: Several optimizations including library purification methods and emulsion PCR titrations were effectively tested. Libraries containing these amplicons were successfully sequenced with median read length exceeding 670bp. Furthermore, up to 80% of the reads were long enough to span more than three consecutive variable regions while up to 50% of the reads contained all 4 amplified variable regions.
Conclusion: The combination of multiple variable regions in one single read leads to a better phylogenetic assignment of the observed taxonomic units. The generation of such long amplicon reads using a high throughput sequencing platform enables a more precise insight into the analyzed community thus opening new perspectives for metagenomic analyses.

GMP010

Mining for novel bacterial chitinases - from chitin-agar plate to screening of metagenomes

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Chitin is one of the most abundant biopolymer on Earth. Although bacteria do not produce chitin, they use it as a source of nitrogen and carbon. The majority of bacterial chitinases are assigned to family18 of the glycosyl hydrolases, in particular to type A encoded by *chiA*. The main objective of this study was to assess the chitin degradation potential of the microbial communities in different terrestrial and aquatic habitats. We examined the bacterial communities of ten different habitats, including aquatic and terrestrial ones. In addition, two large metagenomic fosmid libraries were screened for the presence of chitinase genes. In order to assess the abundance and diversity of chitinolytic bacteria we used enzymatic assays, quantitative PCR, DGGE and 454 deep sequencing based on *chiA*. Clear differences were observed in the abundance of *chiA* gene expression in different habitats. Combined results of enzymatic assays with metagenomic data reveled divergent patterns of richness/diversity versus function. Our study provides new insights in ecosystem composition in terms of the diversity and function of bacterial chitinases.

GMP011

Deep-sea sediments as source for novel glycoside hydrolases

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Due to the world's increased need for renewable and sustainable fuels and chemicals, lignocellulose, as the most abundant biopolymer on earth, is considered to be a potential feedstock for the production of fuels, chemicals, and energy [1]. Since lignocellulose is mainly composed of cellulose and hemicellulose, recent research has focused on the enzymatic degradation of biomass by glycoside hydrolases. The synergistic action of cellulases and hemicellulases allows the breakdown of polymers into oligo- and monosaccharides, which can be further processed in biorefining applications.
For the identification of novel carbohydrate-active enzymes deep-sea sediments collected in Suruga Bay (Japan) were enriched on a polysaccharide mixture as sole carbon source. DNA was isolated from the enrichment cultures and metagenome sequencing was performed using 454-GS FLX Titanium Pyrosequencing (Roche).
Approximately 1.7 Gb of metagenomic sequences with 52,660 open reading frames were obtained from four sequencing runs. 464 open reading frames coding for glycoside hydrolases were identified from this set of data including about 90 genes encoding putative bacterial carbohydrate-active enzymes involved in lignocellulose decomposition.
Several of these genes were expressed in *Escherichia coli* and the recombinant enzymes were investigated for predicted activities. Many of the proteins display the expected glycoside hydrolase activity and they differ in their enzymatic activity. These enzymes hydrolyze various polysaccharides and are active in a broad temperature range.

[1] Lyko, H., G. Deerberg, and E. Weidner (2009). Coupled production in biorefineries-combined use of biomass as a source of energy, fuels and materials. J Biotechnol, 142(1):78-86.

GMP012

Genome assembly of '*Candidatus* Scalindua brodae'

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Bacteria capable of anaerobic oxidation of ammonium (anammox) have many unique features, such as the production and conversion of hydrazine, an anammoxosome compartment and ladderane lipids. Although their core metabolic pathway is largely resolved [1], many questions still remain. Data mining of the (meta)genomes of anammox bacteria is one of the powerful methods to address these questions or identify targets for further study. The availability of high quality reference data greatly aids such analysis. Representatives of four genera of anammox bacteria have been sequenced, but the genome of the marine '*Candidatus* Scalindua profunda' was difficult to assemble in large contigs[2].
Here we present the draft genome assembly of a second *Scalindua* species, '*Candidatus* Scalindua brodae', obtained from an enrichment culture metagenome. At 4.5 Mb and 40% GC, the draft genome assembly resembles those of other genomes of anammox bacteria. Approximately half of the coding sequences match those of `*Candidatus* Scalindua profunda´ with over 90% identity at the amino acid level, indicating large diversity within the genus. Important anammox genes encoding hydrazine synthase, hydrazine dehydrogenase, nitrate reductase, nitrite reductase and carbon monoxide dehydrogenase were identified. Availability of this second *Scalindua* genome will contribute to further elucidation of the defining features of anammox bacteria through comparative genomics.

[1] Kartal, B, *et al*. Molecular mechanism of anaerobic ammonium oxidation.
Nature 479.7371 (2011)
[2] van de Vossenberg, J, *et al*. The metagenome of the marine anammox bacterium '*Candidatus* Scalindua profunda' illustrates the versatility of this globally important nitrogen cycle bacterium." *Environmental Microbiology* (2012).

GMP013

Functional and phylogenetic analyses of microbial communities derived from German soil samples

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Nucleic acid extraction is a crucial step for phylogenetic, transcriptomic and functional analyses of soil microbial communities. To assess the bias introduced by nucleic acid extraction, several commercial and laboratory-established methods for both DNA and RNA extraction were performed for three different soil samples derived from the German Biodiversity Exploratories (seewww.biodiversity-exploratories.de) and analyzed for their efficiencies. Partial 16S rRNA genes and transcripts were amplified using barcoded primers and sequenced using the Roche FLX system with titanium chemistry. Analyses of the derived data indicate that quality and yield of nucleic acids differ considerably with respect to the applied extraction method and analyzed soil. Several soil samples derived from the German Biodiversity Exploratories were subjected to total RNA extraction and mRNA enrichment. Subsequent cDNA synthesis and sequencing allowed the assessment of soil microbial expression profiles.
Metagenomic large-insert libraries were constructed using genomic DNA extracted from the different soil samples. Comparative screening of the libraries for cellulolytic and hemicellulolytic activity was performed, yielding 6 clones expressing the desired activity. Genes encoding hemicellulolytic and cellulolytic activity were recovered from the corresponding clones and sequenced. So far, analyzed hemicellulolytic and cellulolytic enzymes were assigned to glycoside hydrolase families (GHF) 9, 10 and 11, representing one cellulase (GHF 9) and five hemicellulases (GHF 10, 11). Biochemical characterization of hemicellulolytic and cellulolytic enzymes was carried out.

GMP014

Functional metagenomics: Seeking CO_2-reducing enzymes using newly established screening approaches

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Autotrophic organisms have the ability to synthesize organic matter from inorganic carbon sources. Since this transformation provides the grounds for all heterotrophic organisms, the process of autotrophic carbon fixation can be defined as fundamental for the global carbon cycle and for all life on earth. Besides the well-established Calvin-Benson Cycle, five other different autotrophic carbon fixation pathways are known to date. These pathways have been elucidated for isolates. However, little is known about CO_2 fixation pathways of the vast majority of uncultured microbes. Thus, culture independent, metagenomic approaches can offer valuable clues to expand the established knowledge e.g. by recovering new kinds of key enzymes of autotrophic carbon fixation pathways.
The scope of this project is the identification of novel CO_2 reducing enzymes by function based approaches using metagenomic libraries. Since hydrothermal deep sea systems are model habitats for supporting autotrophic growth, we constructed several metagenomic libraries from hydrothermally influenced habitats. One e.g. is from a massive sulfide chimney at Sisters Peak (5° S on the Mid-Atlantic-Ridge). This library consists of 22,000 fosmid clones with an average insert size of 30-35 kb and an insert rate of 99 %. We established two enzyme assays to screen this library for key enzymes of autotrophic carbon fixation pathways.
Our first screen is a colorimetrical one which enables us to detect the reduction of CO_2 to CO, a reaction which is catalyzed by the key enzyme of the reductive acetyl-CoA pathway, the carbonmonoxide dehydrogenases (CODH). The second screen is for assigning the specific activity of the key enzyme of the Calvin Cycle, the ribulose-1,5-bisphosphate carboxylase (RubisCO), an enzyme class which attaches CO_2 to ribulose-1,5-bisphosphate to produce 3-*D*-phosphoglycerate. The RubisCO assay is monitored by using High Pressure Liquid Chromatography (HPLC).
Both screening methods were successfully established and are now used to seek unknown types of CO_2 reducing enzymes from our metagenomic libraries. Using the functional screening approach we already have identified a RubisCO active fosmid clone. Further experiments will verify this activity and characterize the recombinant enzyme.

GMP015

Microevolution of and habitat adaptation of *Pseudomonas aeruginosa*

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Colonisation of environmental or clinical habitats as well as persistence by *P. aeruginosa* goes along with mutation events in the bacterial genome and phenotypical changes. In order to analyse this microevolution, *P. aeruginosa* isolates belonging to the same clonal lineages were sequenced for comparative genomics. Comparisons were done for sequential isolates from cystic fibrosis (CF) patients, collected from the onset of chronic lung infections for up to 20 years, and for clonal variants from different habitats in order to define the genomic background of fitness alterations and other phenotypic traits.
Sequential isolates from CF patients' lungs showed different levels of intraclonal diversity. A clone PA14 lineage acquired only 15 nucleotide substitutions and a large genomic deletion during the observation period and diversified into three branches. A clone C lineage, however, remained invariant over three years before acquiring 959 substitutions and diversifying into multiple branches in the following years. The increased microevolution rate was apparently promoted by establishing a mutation in the *mutL* gene.
Intraclonal diversity of isolates from different sources was analysed for strains belonging to lineages TB and CHA. The TB strains were isolated within close time at Hannover Medical School from CF and burn wound patients. The diversity of these temporally and spatially closely related isolates was very low, only a few nucleotide exchanges and insertion and deletion events were observed. Nevertheless they caused drastic differences in virulence, deletion events in *pil* genes likely triggered global changes in the bacterial behaviour far beyond pilus assembly. Genomic characteristics of the clone TB lineage likely predisposed it for such events. In contrast, spatially different clone CHA isolates from patients and from the environment had diversified much more. Many unique nucleotide exchanges and individually composed accessory genomes reflected the extended specific evolution and habitat adaptation. Although one CHA isolate displayed high virulence potential as well, this trait was apparently not caused by a single microevolution event by a number of successive events.

GMP016

The complete genome analysis of the broad host range strain *Sinorhizobium fredii* USDA257 reveals a wealth of secretion and cell-cell communication-related genes

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Sinorhizobium fredii USDA257 is a Gram-negative and symbiotic nitrogen-fixing bacterium, which was originally isolated from wild soybean cultivars. We have now established the genome of this broad host range strain [1]. Its genome encodes 6,854 ORFs and consists of a single chromosome, cUSDA257 comprising 6.4 Mb (62.09% G+C), and a single symbiotic plasmid, pUSDA257 with an approximated size of 0.5 Mb (59.53% G+C). USDA257 has an extremely broad host range nodulating 79 plant genera [2]. Only the closely related *S. fredii* strain NGR234 reveals a wider host range, being able to nodulate 112 legume plant genera and even one non-legume [2]. The analysis and a detailed comparison of the USDA257 genome with NGR234 revealed a high degree of synteny within these two microbial genomes. Strikingly, cUSDA257 shows almost the same size as cNGR234 and pNGR234*b* together, with high similarities on sequence level. This is in contrast to both symbiotic plasmids, which are very alike in size and genetic contents.
Similar to the NGR234 genome the USDA257 genome encodes for a wealth of secretion-associated genes [3]. It encodes for two complete sets of T3SSs, one located on the chromosome and one on pUSDA257. This makes USDA257 together with NGR234 the only known rhizobial strains carrying two copies of T3SSs. Unlike NGR234, a complete cluster of T6SS related

genes could be identified in USDA257. Surprisingly, and also in contrast to NGR234, USDA257 does not encode a functional T2SS. In total USDA257 carries 152 genes that are putatively linked to secretory processes.
Another highlight of the USDA257 genome lies in the presence of many quorum sensing and quorum quenching-related genes. The USDA257 genome encodes for two autoinducer synthase genes and their respective regulators, most likely involved in the synthesis of *N*-acyl-homoserine autoinducer-like molecules. In addition USDA257 carries at least five gene loci which are linked to the quenching of autoinducer molecules.
Altogether these findings suggest that protein secretion and cell-cell communication are of crucial importance for host-microbe interaction and possibly host range.

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3. Schmeisser C, et al. 2009. AEM, **75(12)**: 4035-45

GMP017
Identification of Gene Clusters for the Biosynthesis of Bromotyrosine derivatives in Metagenomes of the Marine Sponges *Ianthella basta* and *Aplysina cavernicola*

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Marine sponges (*Verongida*) are able to produce a set of bioactive molecules. Among these compounds are bromtyrosines and bromotyrosine derivatives. Bromtyrosines (Bts) are known to have pharmacological relevance. In marine sponges, Bts are typically located within the sponging/chitin based skeleton. They are suggested to protect the chitin skeleton from degradation, through chitinase inhibition. Bts from the species *Ianthella basta* and *Aplysina cavernicola* have already been detected. Beside chitinase inhibition several effects of Bts, such as cytotoxic, antibacterial and anti-inflammatory activities, are known[(1)(2)]. From other biosynthetic pathways, for example the biosynthetic gene cluster of the peptide antibiotic balhimycin, it is known, that halogenation of tyrosine derived residues is catalysed by flavin-dependent halogenases[(3)]. It should thus be possible to detect the Bt-biosynthesic gene cluster of *I. basta* and *A. cavernicola* by using a degenerated PCR primer pair which is specific for flavin-dependent halogenases. Sponges are known to be associated to a large amount with bacterial symbionts[(4)]. Therefore, it seems quite likely that the bromotyrosine producer is rather a bacterial or fungal symbiont than the sponge itself. Thus a symbiont enrichment step was used prior to DNA extraction. Fragments of genes with high homology to flavin-dependent halogenases were found in the metagenomic DNA of both sponges via degenerated primer PCR. The complete sequence of the detected halogenase genes will be obtained, followed by cloning and heterologous expression of the halogenase genes. The flavin-dependent halogenases will be characterised with respect to their halogenating activity and substrate specificity. Finally, we will clone the complete biosynthetic gene clusters by using a metagenomic DNA library.

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(2) Thoms C et al. (2004) Z Naturforsch C 59, 113-122
(3) Pelzer S et al. (1999) Antimicrob Agents Chemother 43, 1565-1573
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GMP018
Development of methods for the discovery of novel lipases from metagenomic datasets

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Lipases are versatile enzymes and key players in the industrial enzyme sector. Whilst accepting a broad range of substrates they are able to maintain a high regio- and enantioselectivity. Due to their high stability in organic solvents lipases can be used for esterification, transesterification, aminolysis and oximolysis reactions in organic synthesis. These properties make lipases useful enzymes and the search for novel lipases a worthwhile endeavor. The marine metagenome with its vast amount of genes from non-culturable bacteria seems to be an ideal prospect for such a search. Instead of using the common approach of screening gene libraries derived from environmental samples we are using bioinformatic methods like profile hidden Markov models to find novel lipases from metagenomic datasets. These profile hidden Markov models give us a probability based scoring system, which we can modify to best represent the primary structure of the proteins we are looking for. We have now developed an *E. coli* expression system to screen and characterize the potential lipases from marine metagenomic databases. This allows us to express codon optimized synthetic lipase genes in *E. coli*. Our vector systems allow for periplasmic targeting and overexpression of putative lipases in *E. coli*, which can then be tested for lipolytic activity on agar plates containing tributyrin as well as agar plates containing triolein and rhodamin b. Tests for substrate selectivity and dependency on specific temperatures and pH-values can be performed using *p*-nitrophenyl esters with acyl chains of different length, in our system. We present here a new and efficient approach to mine the mostly unexplored metagenome for novel enzymes and study them *in vivo* and *in vitro*.

GMP019
Phylogenetic and functional diversity of soil prokaryotic communities in temperate deciduous forests with different tree species

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The soil is a primary habitat for bacteria and archaea. The wide range of properties such as soil pH, texture and nutrient supply provides a multi-layered environment with several niches which diverse prokaryotic communities can colonise. In addition, it is easily accessible as a source of biotechnologically relevant microorganisms.
The advent of metagenomics, and the more recent metatranscriptomics, has led to the exponential growth of phylogenetic data available on microogarnisms. With the development of high throughput sequencing techniques used in platforms such as 454 pyrosequencing, Illumina and SOLiD, massive parallel sequencing of environmental DNA can be achieved at low cost in a relatively short time.
In this project, environmental DNA has been collected from the Hainich National Park in Thuringia, Germany. The sample area consists of mono cluster and mixed cluster tree stands of lime (*Tilia* sp.), hornbeam (*Carpinus* sp.), beech (*Fagus* sp.) and oak (*Quercus* sp.). Samples will be collected over two years in Spring, Summer and Autumn. Changes in prokaryotic community composition with respect to tree species are monitored by amplicon-based sequencing of 16S RNA genes. Analysis of the metatranscriptome is carried out to determine the proportion of active communities in relation to the entire prokaryotic community. Such data provides an insight into the processes taking place in soil habitats, such as nitrogen cycling performed by nitrogen oxidising bacteria and archaea.

GMP020
Characterization of Novel Thiol-Disulfide Oxido-reductases

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Thiol-disulfide oxidoreductases play an important role in different cellular processes, such as redox signaling and protein folding. We are interested in the identification and characterization of novel thiol-disulfide oxidoreductases from the Global Ocean Sampling project (GOS), the largest metagenomic project to date. To find new thiol-disulfide oxidoreductases, we use the power of *Escherichia coli* genetics. *E. coli* has two distinct cellular compartments, the cytoplasm and the periplasm. Three important types of thiol-disulfide oxidoreductases with specific and opposite functions can be found in these compartments. Within the periplasm, the oxidase DsbA is responsible for the oxidation of protein thiols and the periplasmic isomerase DsbC folds substrates correctly by rearranging disulfide bonds. Within the cytoplasm the reductase TrxA keeps protein thiols reduced. In our approach we exploit the fact that phenotypes of null-mutants in genes encoding those proteins can be complemented by oxidases, isomerases and reductases, respectively. We successfully constructed two complementation plasmids, one with an OmpA signal sequence for periplasmic targeting of the protein of interest (pPC) and one for the cytoplasmic targeting which contains no leader sequence (pCC). Additionally, we constructed a plasmid for overexpression (pOE). Phenotypic experiments and protein expression tests revealed correct functionality of the vector systems. With the help of these vector systems we were able to characterize a DsbA-like protein (DLP) and a DsbC-like protein (ILP) phenotypically and biochemically. We present here a system which allows us to study the function of proteins from environmental samples both *in vivo* and *in vitro*, thus opening the door to functional metagenomics.

GMP021

Heterologous production of novel class II lantibiotics in *E. coli*

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The rapid spread of multidrug resistance in clinically relevant pathogens necessitates the search for novel antibiotics. Lantibiotics, a family of ribosomally synthesized bacterial peptide antibiotics, are promising leading structures for the development of future therapeutics. A common property of this peptide family is the presence of the rare thioether amino acids lanthionine and methyl-lanthionine. These amino acids are introduced by specific modification enzymes by means of the dehydration of Ser/Thr followed by reaction of the resulting dehydro amino acids with cysteines.
Blast searches employing known lantibiotic biosynthesis enzymes (LanM) in the NCBI database revealed that ORFs coding for proteins involved in lantibiotic production are widespread in bacteria of different phyla. Based on these data, we identified new class II gene clusters in different bacterial strains such as in *Bacillus pseudomycoides*, *Bacillus licheniformis* and *Caldicellulosiruptor bescii*. We additionally found putative structural genes (LanA) in metagenomic sequences e.g. obtained from the human gut or vaginal flora.
Our project focuses on the characterization of new lantibiotics by heterologous expression of predicted biosynthetic genes (LanA, LanM) in *E. coli*. In addition to the biosynthesis machinery of the well-known lantibiotic mersacidin, putative lantibiotics that display interesting peptide characteristics e.g. repetitive motifs, extremely long leaders or propeptide sequences, were included in our project.
Coexpression of a lantibiotic structural gene found in *Bacillus pseudomycoides* along with its corresponding modification enzyme resulted in the production of a modified prepeptide with 4 dehydrations. After the proteolytic removal of the leader sequence *in vitro* by the protease factor Xa, the heterologously produced prepeptide showed an antimicrobial activity against the indicator strain *Micrococcus luteus*. Additionally, the molecular weight of the peptide isolated from *E. coli* corresponded to a mass signal present in antimicrobially active cell extracts of the producer strain *B. pseudomycoides*.
In summary, we were able to heterologously produce the novel lantibiotic pseudomycoidicin in *E. coli*. Following the same strategy, production of other lantibiotics predicted in sequenced genomes are in the focus of our on-going work.

GMP022

Mapping of a putative roseoflavin biosynthesis gene cluster in *Streptomyces davawensis* and initial characterization of a novel early intermediate

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Streptomyces davawensis synthesizes the antibiotic roseoflavin, a structural riboflavin (vitamin B_2) analog. Roseoflavin exhibits antibiotic activity against Gram-positive and also Gram-negative bacteria if it is able to enter the cell. It was postulated earlier that roseoflavin is synthesized from riboflavin *via* 8-amino-8-demethyl-riboflavin and 8-methylamino-8-demethyl-riboflavin. In our laboratory a novel *N,N*-8-amino-8-demethyl-D-riboflavin dimethyltransferase (RosA) was identified, catalyzing the two terminal steps of roseoflavin biosynthesis. An *S. davawensis* expression library with an insert size of approximately 100kbp was constructed in *Escherichia coli* using a modified version of pPAC-S1. The latter library was screened by PCR for a strain harboring *rosA*. The corresponding PAC (with a 100 kbp *S. davawensis* subgenomic insert) was isolated and transferred to *Streptomyces coelicolor* M1152 by conjugation. A resulting exconjugant strain synthesized roseoflavin, which strongly suggests that all genes necessary for heterologous production of roseoflavin were located within this 100 kbp region. Inactivation of the CDS sdav79780 (encoding a putative beta-lactamase domain-containing protein) located in the 5'-region of the *rosA* containing PAC led to the accumulation of a yet unknown compound. Mass spectrometry revealed that the latter compound is a riboflavin derivative and thus very likely is an intermediate of roseoflavin biosynthesis. We are currently purifying this novel compound from a large scale culture and plan to determine the molecular structure by NMR.

GMP023

Metatranscriptomic analysis of microbial communities in the oxic and anoxic zones of flooded paddy soil

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Flooded rice fields are one of the major biogenic sources of atmospheric methane. In this study, we analyzed and compared the metatranscriptome of microbial communities inhabiting the oxic surface layer and anoxic bulk soil of flooded rice paddy soil microcosms incubated in the greenhouse under normal day/night cycles. Samples for metranscriptomic analysis were taken at three different time points, corresponding to the different growth stages of the rice plant: tillering, flowering, and ripening.
Successional changes in microbial community composition during plant growth were monitored by massively parallel sequencing of 16S rRNA-tags, randomly produced from total RNA. Microbial activities at the ripening stage were assessed by analysis of mRNA enriched by subtractive hybridization of rRNA.
The microbial consortia in the oxic surface layer showed no major changes in community composition over the different growth stages of the rice plant and were dominated by cyanobacteria (40-55% of total 16S rRNA tags). In the anoxic zone, microorganisms typically involved in the anaerobic degradation of organic matter, such as *Geobacter, Anaeromyxobacter, Clostridia,* and methanogens, were prevalent. Their relative abundance gradually increased during plant growth.
Essential genes for habitat-specific functions, e.g, light-harvesting complexes in photosynthesis and methyl-coenzyme M reductase in methanogenesis, were expressed exclusively in the oxic and anoxic zone, respectively. Differential expression patterns were observed for genes related to central carbohydrate metabolism. Transcripts encoding enzymes of TCA cycle, including various dehydrogenase complexes for anaplerotic reactions, were overrepresented in the oxic surface layer, while transcripts involved in pyruvate metabolism II (i.e., acetate formation via acetyl-CoA) were significantly more prevalent in the anoxic zone.
In summary, metatranscriptomic analysis revealed distinct functional activities in response to differences in the physico-chemical conditions that occur in the oxic surface layer and anoxic bulk soil.

GEOV001

Sulfur cycling associated with the anaerobic oxidation of methane

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Anaerobic oxidation of methane coupled to sulfate reduction (AOM) is a major process responsible for controlling the emissions of methane, a potent greenhouse gas, from marine sediments. Sulfate-coupled AOM is believed to be performed by a microbial consortium of methanotrophic archaea (ANME) and sulfate-reducing Deltaproteobacteria [1] but the underlying mechanism remains a geomicrobiological puzzle [2].
We investigated sulfur cycling associated with AOM in a microbial enrichment culture. We used a combination of cultivation approaches, isotope labeling experiments and single-cell-based techniques, such as Raman micro-spectroscopy and nanoscale secondary ion mass spectrometry. Based on our results we proposed a new model for marine AOM, in which both methane oxidation and sulfate reduction to zerovalent sulfur is performed by the methanotrophic archaea [3]. Furthermore, we could show that the associated Deltaproteobacteria disproportionated the produced sulfur in a form of disulfide to sulfate and sulfide.
These new observations expand the physiological diversity of known microbial sulfur metabolisms. Moreover, our results propose that zerovalent sulfur plays a key role in AOM, which has important implications for biogeochemical carbon and sulfur cycling in marine sediments.

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GEOV002

Hydrogen and acetate as electron donors for microbial manganese reduction in a manganese-rich marine sediment

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Energetically, manganese oxide is a highly favorable electron acceptor, and recent studies showed that with available manganese oxide microbial manganese reduction can dominate carbon oxidation over iron and sulfate reduction in marine sediments. We aimed to investigate the electron donor usage and identity of manganese-reducing bacteria in manganese oxide-rich surface sediment of Gullmar Fjord (Sweden) in comparison to the well-studied groups of iron and sulfate reducers. Fermentation products like hydrogen and acetate are expected to be the most important electron donors for manganese reduction in analogy to iron and sulfate reduction. The role of these substrates was investigated in anoxic incubations from surface sediment (0-5 cm) over 30 days which were completely dominated by manganese reduction as terminal electron-accepting process. The average hydrogen concentration was 0.02 nM during the incubation and thus, significantly lower than in incubations dominated iron and sulfate reduction as electron-accepting process. ^{14}C-acetate turnover rates were 1.5-7.1 nmol cm^{-3} d^{-1} and acetate oxidation contributed 4-14% to anaerobic carbon oxidation in manganese reduction-dominated incubations similar to incubations with iron and sulfate reduction as terminal electron-accepting processes. In addition we identified the acetate-oxidizing manganese-reducing bacteria by 16S rRNA-stable isotope probing (SIP) as members of gammaproteobacterial *Colwellia* and *Oceanospirillaceae* as well as epsilonproteobacterial *Arcobacter*. In conclusion i) hydrogen concentrations were thermodynamically controlled by manganese reducers, ii) acetate was used but not the most important electron donor for manganese reduction and iii) the identified acetate-oxidizing manganese reducers differed from the well-known groups of iron reducers.

GEOV003

Signatures of endosymbiosis in recent and fossil bivalves

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Endosymbionts in marine bivalves leave characteristic biosignatures in their host organisms. We detected isotopic signatures of recent chemosynthetic and also photosynthetic bivalve species and were able to trace back these signatures to subfossil samples. As an example, the symbiont-bearing bivalve *Loripes lacteus* was studied in detail and compared with the filter-feeding *Venerupis aurea* (without symbionts). Both bivalves were taken from the same sampling sites in Mediterranean lagoons. We analyzed the isotopic composition of shell lipids (δ^{13}C) and the bulk organic matrix of the shell (δ^{13}C, δ^{15}N, δ^{34}S). In the thiotrophic *Loripes*, δ^{13}C values were depleted compared with the filter-feeding *Venerupis* by as much as 8.5 ‰ for some fatty acids and 4.4 ‰ for bulk organic carbon. Likewise, bulk δ^{15}N and δ^{34}S values were more depleted in thiotrophic *Loripes*. These features were compared with data from subfossil samples. Whereas δ^{34}S values were found to be unstable over time, the combined δ^{15}N and δ^{13}C values in organic shell extracts revealed a specific signature for chemosymbiosis in recent and subfossil specimens. This method allows us to study feeding habits of fossil specimens, even of extinct species.

Dreier, A. et al., (2012) *FEMS Microbiol. Ecol.* 81, 480-493.

GEOV004

Tracing seasonal and spatial diversity and activity of marine Thaumarchaeota using intact polar lipids and gene expression

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Thaumarchaeota are ammonia-oxidizing Archaea (AOA) often abundant in aquatic ecosystems. The aim of this study was to evaluate the presence and role of AOA in the nitrogen cycle in marine sediments, which is less clear than in the marine water column. We analyzed crenarchaeol, a lipid specific for this group, in the form of intact polar lipid (IPL, marker for living organisms) in the water column and sediment cores in the North Sea (transect from coast to central). The lipid data was complemented by DNA (abundance) and RNA (activity) analysis of thaumarchaeotal 16S rRNA and metabolic genes.

Differences in the thaumarchaeotal population were observed between different locations, depths and seasons showing a clear difference in the abundance and activity between water and sediment compartments. Thaumarchaeota abundance, based on lipid and DNA quantification, did not change between seasons but the microbial activity was higher in the winter. This was likely associated to a higher availability of oxygen deeper in the sediments during the winter, as well as more availability of ammonium derived from summer algal blooms sinking down in the water column. Thaumarchaeotal abundance also varied between different stations in the North Sea and higher values were observed in areas of higher deposition of particulate matter. A high relative abundance of HPH-crenarchaeol and activity was detected in sediment cores up to 12 cm depth, which indicates the presence of an active living population of Thaumarchaeota in areas with oxygen levels under the detection limit.

This combined approach provides information on the diversity, abundance, niche and seasonality of AOA, which can help to evaluate the role of this group in the global carbon and nitrogen cycles.

GEOV005

Cold volcanic CO_2 emanations in a wetland area promote *Acidobacteria*, acetogens and methanogens

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We investigated mofettes, cold Volcanic CO_2 exhausts, in a wetland area in the NW Czech Republic. Here, continous emanations of CO_2 lead to lower pH and anoxic conditions throughout. Recent findings suggest that such alteration might cause a shift of the microbial community towards anaerobic and acidophilic organisms.

In this study we i) analyzed differences in the active archaeal and bacterial community structure in different depths of a mofette compared to the surrounding wetland soil by 16S rRNA pyrosequencing and ii) combined DNA- and PLFA-based ^{13}C-CO_2 Stable Isotope Probing (SIP) as well as carbon stable isotope analysis of pore water acetate and methane to identfy microbial communities which can incorporate the emanating CO_2.

16S rRNA pyrosequencing revealed that the overall active bacterial community composition was similar for the mofette and reference soil in all sampled depths. However, in the mofette soil *Acidobacteria* showed a higher relative contribution (46% to 65%) compared to the wetland reference (16% to 19%) primarily consisting of one OTU closely related to Subdivision 1 isolate „Ellin 624“ (98% Sequence Identity). The active archaeal community of the mofette soil was dominated by methanogens in all depths which were not represented in the wetland reference soil.

Analysis of incorporation of ^{13}C-labelled CO_2 into PLFAs and ether lipid derived hydrocarbons in the mofette soil showed continous enrichment over 28 days in almost all bacterial and archaeal biomarkers. While latter indicated that archaeal CO_2 utilization is restricted to the first 10 cm of the soil a depth independent labelling pattern was found for bacterial biomarkers. This finding was supported by labelled 16S rRNA gene analysis of the first 10 cm which showed that members of almost all present major bacterial phylums incorporated ^{13}C-CO_2. Phylogenetic analysis of labelled formyl tetrahydrofolate synthetase (*fhs*) gene sequences revealed two novel groups of acetogens within the *fhs* cluster A. Together with pore water data, these findings suggest that acetogens and methanogens profit from the upstreaming CO_2 and the lowered pH seems to favor specifically one subgroup of the *Acidobacteria*.

GEOV006

Elucidation of microbial communities involved in CO_2 fixation in karstic limestone aquifers targeting RubisCO- and ammonia-monooxygenase encoding genes

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In oligotrophic limestone aquifers, chemolithoautotrophy may play an important role in the overall carbon flow. Here, we investigated the CO_2-fixing bacterial communities in the groundwater of a shallow, suboxic, and a deep, oxygen-rich limestone aquifer in the Hainich region (Thuringia, Germany), targeting *cbbL* and *cbbM* genes encoding RubisCO type I and II. In addition, we studied the microbial communities involved in ammonia oxidation, the first step of nitrification, targeting *amoA* genes encoding ammonia mono-oxygenase. The objectives of this study were (i) to follow seasonal dynamics of the CO_2-fixing and the ammonia-oxidizing communities over a two years period, (ii) to analyze their diversity targeting *cbbM, cbbL,* and *amoA* genes and transcripts, and (iii) to elucidate links between autotrophy and nitrification. Results of quantitative PCR suggested that approximately 0.3 to 14 % of the groundwater bacterial population had the genetic potential to fix CO_2 via the Calvin Cycle with strong seasonal fluctuations but without significant differences between the two aquifers. In contrast, the abundance of ammonia oxidizers was clearly linked to oxygen availability with significantly higher *amoA* gene copy numbers in the deeper aquifer. Pyrosequencing of *cbbM* transcripts revealed that the active CO_2-fixing bacterial communities were dominated by *Thiobacillus* sp. related organisms in both aquifers. Analysis of *cbbL* genes and transcripts showed that *Nitrosomonas ureae*-related phylotypes constituted a substantial fraction of the CO_2-fixing community in the deep aquifer, which was confirmed by *amoA* gene-based analysis. Our data provide strong evidence that chemolithoautotrophy coupled to nitrification contributes to the carbon flow in a limestone aquifer at high oxygen availability while carbon fixation appears to be primarily linked to other metabolisms involving sulphur and iron under low oxygen availability.

GEOV007

Natural CO_2 vents affect freshwater environment

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The Laacher See volcanic centre, located in the middle of the East Eifel volcanic field, west of the river Rhine, discharges about 5 000 t CO_2 per year. The CO_2 is released from multiple gas vents at the bottom of the lake. Natural CO_2 sources like Laacher See allow the determination of CO_2-induced biological and geochemical alterations of surface ecosystems. Therefore, microbial metabolisms, abundance and diversity together with related biogeochemical parameters were studied to assess potential effects of elevated CO_2 concentrations on freshwater sediment ecosystems.
CO_2 seeps at the lake bottom and reference areas were localised using different hydroacoustic measurements. The flux rates and the composition of seeping gases were verified with divers and a small remotely operated vehicle (ROV). For the investigation of active metabolic pathways, cultivation experiments under aerobic and anaerobic conditions were conducted and the formation of CO_2 and CH_4 was analyzed by gas chromatography. The microbial population was characterized using quantitative real time PCR (qPCR) for 16S rRNA and functional genes, TRFLP and sequencing.
Dissolved CO_2 in bottom water as well as in sediment pore water samples had a carbon isotopic signature close to that in the gas bubbles, both confirming a magmatic origin of the gas. Analysis of water samples collected close to intensive CO_2 seeps showed a low pH and an increase of dissolved CO_2. Furthermore, geochemical and microbiological analyses of up to 2 m long sediment cores from CO_2-affected and reference sites showed alterations in pH, microbial activity and populations. 16S rRNA gene copy numbers of *Bacteria* and *Archaea* from CO_2 induced and reference sites varied by four orders of magnitude. Similar results could be detected for the analyzed microbial CO_2 and CH_4 turnover. Both, methane and CO_2 production rates decreased with increasing CO_2 concentrations. Our results illustrate a CO_2 induced shift in microbial activity and community composition caused by the increasingly anaerobic and acidic environmental conditions.

GEOV008

Predicting the metabolic potential of a novel mining associated iron oxidizing bacterium by comparative genomics

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Recently, iron oxidizing bacteria of the novel putative genus "*Ferrovum*" have been detected in a remediation plant of acid mine drainage water in Lusatia (Saxony, Germany). Due to the tenacious association between "*Ferrovum*" and *Acidiphilium* sp. it is difficult to obtain a pure culture - similar observations have been made for the acidophilic iron oxidizer *Acidithiobacillus ferrooxidans*. Since the physiology of "*Ferrovum*" cannot be investigated in the mixed culture we used a genomic approach to, firstly, characterize the lifestyle of "*Ferrovum*" and the reasons for its dominating abundance in the pilot plant. Secondly, we wanted to understand possible interactions between "*Ferrovum*" and *Acidiphilium* which might be responsible for their close association.
Using comparative genomics we were able to identify the proteins involved in the pathways of the central metabolism in "*Ferrovum*". Now we can describe robust models for the uptake and assimilation of the essential nutrients C, N, P, and S. In this context we also revealed a gene cluster encoding a putative urea ABC transporter and the urea hydrolyzing enzyme urease - thus possibly enabling "*Ferrovum*" to use urea as an additional nitrogen and carbon source. Nevertheless the genome analysis was restricted in respect of the prediction of the proteins involved in the iron oxidation. Theoretically, "*Ferrovum*" transfers electrons from ferrous iron either to oxygen to produce ATP or, to $NAD(P)^+$ to generate NAD(P)H. Interestingly, the "*Ferrovum*" genome does not encode the proteins rusticyanin or the iron oxidase that play an important role in the iron oxidation in other iron oxidizers. However, the "*Ferrovum*" genome encodes several uncharacterized cytochromes and ferredoxins that may be involved in the iron oxidation.
In order to explore the relationship between "*Ferrovum*" and *Acidiphilium* we analyzed the genome with focus on their carbon metabolism. Here we present a model of the potential interspecies carbon flow: „*Ferrovum*" fixes carbon dioxide via the Calvin cycle to produce biomass, such as cell envelope polysaccharides, and therefore provides an organic carbon source for *Acidiphilium*. Some of these polysaccharides are then converted back to carbon dioxide which, in turn, increases the local carbon availability for "*Ferrovum*".

GEOV009

Electron density modelling for the fate prediction of halogenated heteroaromatics in anaerobic environments

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Heterocyclic aromatic compounds like polyhalogenated dibenzodioxins or dibenzofuranes are among the most toxic compounds known and tend to accumulate in the environment. Under anaerobic conditions, specialized bacteria of the genus *Dehalococcoides* can detoxify these compounds by removing halogens in a respiratory process [1, 2]. For predicting the fate of the various halogenated aromatic compounds in anaerobic environments as well as for assessing the scope of a biotechnical use of *Dehalococcoides* species for bioremediation, a deeper understanding of the dehalogenation reaction is necessary.
Here we show how non-halogen substituents and heteroatoms including oxygen, sulphur and nitrogen affect the ease of removal of halogen substituents from aromatic compounds by *Dehalococcoides* strain CBDB1. Strain CBDB1 was cultivated with a wide variety of brominated and chlorinated compounds such as benzonitriles, anilines, methoxylated and hydroxylated benzoic acids, furoic acids, thiophenes, pyridines and phenols. Compounds containing nitrogen as substituent or heteroatom were poor

substrates, while sulphur and oxygen containing substituents or heteroatoms enhanced reductive dehalogenation. Growth yields of $1x10^{13}$ to $5x10^{14}$ cells per mol of halogen released were obtained. For all tested halogenated aromatics and their reaction products, Hirshfeld partial charges were calculated employing the quantum chemical density functional theory, and applied to establish a model that allows predicting the susceptibility of compounds with respective heteroatoms and substitution patterns to undergo reductive dehalogenation. Overall, the experimental data and calculated net atomic charges provide evidence for a new reaction mechanism of reductive dehalogenation catalyzed by strain CBDB1.

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GEOV010
Chemolithoautotrophic nitrate-dependent Fe(II) oxidizing nature of members of genus *Thiomonas*

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Microbial Fe(II) oxidation in anoxic environments has been shown to be mediated by coupling to denitrification processes. Geochemical studies conducted on a diverse range of soils have reported evidence of this physiological process and also indicated that it could be performed by autotrophically growing microorganisms. Although several studies have shown the widespread nature of chemolithoautotrophic nitrate-dependent Fe(II) oxidation, this physiological process is not well understood due to the lack of pure cultures. Attempts to isolate these organisms have led to some bacterial isolates which were shown to be incapable of autotrophic growth after successive sub-culturing. Lake Grosse Fuchskuhle is an acidic bog lake, which was shown to have a low methanogenic potential possibly due to a dominant Fe(III) reduction process in the sediment. Studies have also shown the presence of *chemolithoautotrophic nitrate-dependent Fe(II) oxidizing microorganisms* in this sediment and hypothesized that these microorganisms could be mediating the rate limiting step of regenerating Fe(III) by Fe(II) oxidation for sustainment of continuous Fe(III) reduction. The present study was conducted on the littoral sediment of Lake Grosse Fuchskuhle with the objective of enriching and characterizing *chemolithoautotrophic nitrate-dependent Fe(II) oxidizing microorganisms*. The enrichment incubations indicated that members of the genus *Thiomonas* could be capable of *nitrate-dependent Fe(II) oxidation*. Further characterization of the *Thiomonas* demonstrated a stoichiometric consumption of Fe(II) and nitrate required for nitrate-dependent Fe(II) oxidation. Stable isotope probing using $^{13}CO_2$ was performed on the enrichment and showed labeling of these *Thiomonas* species, indicating the autotrophic growth of these organisms under nitrate-dependent Fe(II) oxidizing conditions. Furthermore quantification of RuBisCo (*cbbL*) gene copy numbers by quantitative real-time PCR showed a logarithmic increase of these genes under the incubation conditions. To our knowledge *nitrate-dependent Fe(II) oxidation* in this genus has not been reported before. Moreover in contrast to earlier isolates from this physiological group, these *Thiomonas* species could be successively sub-cultured without losing the capability of autotrophic growth.

GEOV011
Carbon isotope fractionation and the acetyl-CoA pathway

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The fractionation of stable carbon isotopes has been intensively studied for higher organisms (e.g. plants). It can be summarized that only the initial carbon fixation (mainly C_3 and C_4 photosynthesis) discriminates against the heavier ^{13}C carbon isotope. All downstream anabolic as well as catabolic processes show only minor isotopic influences. In general the isotopic signal of carbon in natural environments ranges between inorganic carbonate rocks (~ -0‰) and biologically fixed carbon derived from C_3 plants (~ -35‰). A similar range can be observed for CO_2 fixation into biomass by all the known autotrophic microbial pathways. Like in higher organisms many catabolic processes (e.g. fermentation) show only a very minor discrimination against the heavier ^{13}C. A clear exception is the acetyl-CoA pathway; resulting e.g. in atmospheric methane with -47‰. This pathway is not only used by methanogenic archaea but likewise by homoacetogenic and some sulphate reducing bacteria. Depending on the organism it can be fuelled by different substrates and can be run in both directions. There is growing evidence that especially the incorporation of C_1 compounds is associated with a pronounced fractionation of stable carbon isotopes. In contrast to many other systems it seems that the initial carbon fixation is not the only fractionating step.

GEOV012
Metal interaction processes with cell wall components of Gram-positive bacteria studied by QCM-D

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Bacterial isolates from the uranium mining waste pile Haberland (Johanngeorgenstadt, Saxony) possess high affinities for heavy metals [1]. Binding sites are predominantly provided by the components of the bacterial cell wall, mainly by surface layer proteins, but also from other parts of the cell wall e.g. membrane lipids.
A deeper understanding of the metal interaction with the biosphere is important not only from an ecological point of view but also from an application oriented one.
Parts of our investigations focused on surface layer proteins (S-layers). They represent the outermost cell envelope of many eubacteria and archaea forming highly ordered paracrystalline lattices not only on the living cell, but also after isolation on various technical surfaces by self-assembling processes [2]. Such biological structures can be used e.g. as filter materials for waste water treatment and as templates for synthesis of bio-based sensory layer or chemical catalysts [3]. Nevertheless, the investigation of interactions of the cell wall components like S-layer, peptidoglycan, lipids and secondary cell wall polymers (SCWP) with metals and nanoparticles both as molecules and as intact layers on a molecular level remains challenging.
In addition, to standard bio-analytical methods the quartz crystal microbalance with dissipation monitoring (QCM-D) represents a versatile tool to track and control the biological layer formation, metal interaction and nanoparticle deposition as well as adsorption kinetics. This method allows the real time detection of sorption processes on a molecular level and gives further information of the viscoelasticity [4].
Aim of our study was the investigation of layer adsorption of isolated cell wall components on technical surfaces such as glass or silicon using a simplified model derived from Gram-positive bacteria in order to get basic information about multilevel processes in complex natural systems. The sorption behavior of metals with these components will be investigated by QCM-D. The results were evaluated by supporting atomic force microscopy (AFM).

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GEOP001
Structure and function of microorganisms in the methanic zone of the Helgoland mud area, North Sea

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In many marine sediments, sulfate and methane depletion are coupled giving rise to a sulfate-methane transition zone (SMTZ). Sulfide might diffuse downward from the SMTZ into the methanic zone and react abiotically with Fe (III) minerals buried over geological time scale. This results in the formation of Fe (II) and elemental sulfur which can now be disproportionated into sulfate and sulfide, thereby, fuelling a cryptic sulfur cycle (1). In our samples from the Helgoland mud area, only negligible amounts of sulfate and sulfide was detected beyond 50 cm, whereas Mn^{2+} and Fe^{2+} in pore water still occurred down to 500 cm below the seas floor at concentrations of up to 100 μM and 350 μM respectively. This leaves open the possibility of a microbe-mediated iron (III) reduction. To reveal the possible microbial involvement of iron (III) reduction in the methane zone, Bacteria and Archaea community present therein was characterized by culture-independent molecular analysis. A 500 cm sediment core spanning the SMTZ down to the methane zone was obtained from the Helgoland mud area using a gravity corer (GC) while a 40 cm core was obtained with a

multiple corer (MUC). The gravity core was sectioned in 25 cm interval down the depth while only the top 10 cm (zone of Mn^{4+} and Fe^{3+} reduction) of the MUC was sampled in 5 cm intervals. DNA was extracted from sediments of all depths sectioned and subjected to Terminal Restriction Fragment Length Polymorphism (TRFLP), qPCR and 16S rRNA-, *mcrA*- and *dsrB*-targeted 454 pyrosequencing. Bacteria and Archaea TRFLP profiles obtained from the SMTZ down the core were similar but different from surface sediment profiles. qPCR data based on 16S rRNA gene also revealed that in the Helgoland mud area, Bacteria counts ranged from 10^8 - 10^9 gene copies/g wet sediment while Archaea counts ranged from 10^7 - 10^8 gene copies/g wet sediment throughout the depths. Comprehensive analysis of 454 pyrosequencing data is currently underway.

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GEOP002

The role of humic substances as electron shuttle for iron reduction in marine sediments

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Humic substances (HS) are chemically heterogeneous polymeric organic compounds that are produced by the decay of organic matter [1,2]. They can be found in soils, waters and sediments, and can serve as electron acceptor for the oxidation of organic compounds (e.g. acetate), electron shuttles, or electron donors [3,4,5,6]. The role of HS as electron acceptor and shuttle and their importance in the reduction of metals in soils, fresh water sediments, and pure cultures has been shown by numerous studies. However, the ability to use HS in the marine system and the impact on the iron cycle is rarely being investigated. Here, we have examined the effect of the humic acids on electron shuttling in iron cycle in marine sediments. Sediment samples from Aarhus bay were incubated at 10^0C in the presence of 0.5 mM sodium acetate as electron donor, the humic acid analogue anthraquinone-2-4-disulfonic acid (AQDS, 50 μM) as electron shuttle, and lepidocrocite as supplemented iron oxides, and terminal electron acceptor. Sulfate reduction was controlled by using sulfate-free artificial sea water for preparing slurries. Preliminary results showed that AQDS amendment at low concentrations stimulated iron reduction rates 5-fold over unamended controls. Thus, microbial populations in marine sediments are capable of using electron shuttling compounds *in situ*, thereby enhancing iron reduction. Further experiments are currently underway to investigate this ability in samples from different sites.

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GEOP003

Oxygenic photosynthesis in cyano-bacteria as a protection mechanism against iron precipitation

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We found a mechanism how Cyanobacteria can avoid encrustation by iron oxides. Such a crust can lead to cell death, by hampering the uptake of nutrients, the excretion of waste products and shielding phototrophic bacteria from light. We studied cyanobacteria biofilms growing in illuminated bioreactors at the Äspö Hard Rock Laboratory (Sweden), fed with either iron-rich or iron-poor ground water. Biofilms growing at low Fe^{2+} concentrations (1 μM) were highly encrusted in Fe-oxides and low in biomass. Remarkably, biofilms growing at high Fe^{2+} concentrations (30 μM) were not encrusted and attained high biomass. Oxygen and pH profiles were measured in situ. The cyanobacteria from the high Fe^{2+} environment showed much higher photosynthesis rates than those from the low Fe^{2+} environment. Measurements under controlled laboratory conditions showed increasing photosynthesis rates with increasing Fe^{2+} concentrations (up to 50 μM) for the biofilms originating from a high Fe^{2+} reactor. The biofilms from the low Fe^{2+} reactor decreased their photosynthesis under increasing Fe^{2+} levels. This suggests that the biofilms growing under high Fe^{2+} levels developed a protective mechanism avoiding encrustation: In response to high Fe^{2+} the cells enhance their photosynthesis rates to create a microenvironment conducive for Fe-oxidation (high pH and O_2). We suggest that a high photosynthetic rate is a selective force for phototrophs living in high Fe^{2+} environments. These cyanobacteria, can further modulate their photosynthesis rates to produce an O_2 and/or pH-barrier against iron precipitation on the cell. In this case the Fe^{2+} oxidizes in some distance from the biofilm and can no longer adsorb onto the cell surface.

GEOP004

Elevated pressure of carbon dioxide affects growth of thermophilic *Petrotoga* sp.

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Carbon capture and storage (CCS) is considered a promising new technology which reduces carbon dioxide emissions into the atmosphere and thereby decelerates global warming. During CCS, carbon dioxide is captured from emission sources (e.g. fossil fuel power plants or other industries), pressurised, and finally stored in deep geological formations, such as former gas or oil reservoirs as well as saline aquifers. However, with CCS being a very young technology, there are a number of unknown factors that need to be investigated before declaring CCS as being safe. Our research investigates the effect of high carbon dioxide concentrations and pressures on an indigenous microorganism that colonises a potential storage site.
Growth experiments were conducted using the thermophilic thiosulphate-reducing bacterium Petrotoga sp., isolated from formation water of the gas reservoir Schneeren (Lower Saxony, Germany), situated in the Northern German Plain. Growth (OD600) was monitored over one growth cycle (10 days) at different carbon dioxide concentrations (50%, 100%, and 150% in the gas phase), and was compared to control cultures grown with 20% carbon dioxide. An additional growth experiment was performed over a period of 145 days with repeated subcultivation steps in order to detect long-term effects of carbon dioxide. Cultivation over 10 days at 50% and 100% carbon dioxide slightly reduced cell growth. In contrast, long-term cultivation at 150% carbon dioxide reduced cell growth and finally led to cell death. This suggested a more pronounced effect of carbon dioxide at prolonged cultivation and stresses the need for a closer consideration of long-term effects.
Experiments with supercritical carbon dioxide at 100 bar completely inhibited growth of freshly inoculated cultures and also caused a rapid decrease of growth of a pre-grown culture. This demonstrated that supercritical carbon dioxide had a sterilising effect on cells. This effect was not observed in control cultures with 100 bar of hydrostatic pressure.
Further experiments will examine physiological and molecular properties of the model organism allowing for prediction of its sensitivity and/or adaptability to carbon dioxide in potential future storage sites.

GEOP005

Ecological impact assessment of iron oxide nanoparticles used for bio-remediation of hydrocarbon conta-minated aquifers.

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The aim of the NanoSan project is to enhance microbial remediation of contaminants in groundwater via injection of iron-oxide nanoparticles (NP). It was demonstrated that Fe-oxide NP can strongly enhance microbial iron reduction in pure culture (Bosch et al., AEM 2010). However, little is known about the effect of Fe-oxide NP on natural microbial communities and their potential ecotoxicity. In this study we investigate the effects of iron oxide nanoparticles on iron-reducing BTEX-degraders, as well as the ecotoxicology of selected iron oxide nanoparticles on non-iron-reducing microbes and higher organisms (nematodes) important in the subsurface.
We measured ATP as universal parameter of microbial activity and used cutting edge molecular biology techniques to unravel stimulation vs.

inhibition effects of Fe-oxide NP on distinct microbial community members. ATP production in 1D columns of uncontaminated sediments amended with increasing concentrations of NP revealed a stimulation of overall bacterial activity at concentrations <5.4 mM, and inhibition at >16 mM. Pyrotag analyses indicated a rather stable total bacterial community, and even at high NP concentrations, a loss of diversity or total gene counts was not observed.
In addition, samples taken from a contaminated test site used for demonstration purposes were subjected to a depth resolved initial examination of the microbial communities in the sediments. A surprisingly homogeneous distribution and a generally high similarity of bacterial communities was found over depth. This information will now be the basis for the identification of potential degrader populations *in situ* as well the inference of potential impacts of NP application on overall aquifer microbiota at the site.
Reference: Bosch, J.; Heister, K.; Hofmann, T.; Meckenstock, R. U. Nanosized iron oxide colloids strongly enhance microbial iron reduction *Appl. Environ. Microbiol.* 2010, **76** (1) 184-9

GEOP006

Nanosized iron oxides in microbial iron reduction: electron shuttling along redox gradients and impact of organic matter

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Microbial reduction of ferric iron is a major biogeochemical process in groundwater ecosystems and often associated with the degradation of organic contaminants, as bacteria couple iron reduction to the oxidation of organic molecules like e.g. BTEX. Yet the high crystallinity and low solubility of iron oxides limits reduction rates. However, environmental nanosized iron(oxy)hydroxide minerals seem to have an unequally enhanced reactivity potential compared to their bulk and highly crystalline parent materials of the same mineral.
Here, we examined the reactivity of nanosized, synthetic and environmental colloidal iron oxides in microbial iron reduction in static batch incubations and column experiments. Furthermore, we studied the influence of adsorbed and coprecipitated humic acids on microbial iron oxide accessibility.
Colloidal nanoparticles showed a significant enhancement of reaction rates as compared to macrophases, independent of the kind of mineral phase or surface area. The presence of humic acids accelerated the microbial iron oxide reduction. Coprecipitated humic acids resulted in a similar enhancement as adsorbed humic acids at a range of OC/Fe ratios. Furthermore, soil column experiments demonstrated the high mobility and persistence of nanosized iron oxides under simulated environmental conditions, opening the perspective for their technological application as electron acceptors in the remediation of BTEX and putatively PAH contaminated sites. First studies on the feasibility of this technology showed a 5x-fold enhancement of toluene oxidation after application of nanosized iron oxide particles to microbial incubations, as well as the possibility of custom-tailoring the subsurface mobility of these particles after injection into a contaminant plume.
In summary, our results indicate the importance of a microbial, coupled iron oxide nanoparticle-humic substance electron shuttling system along redox gradients in groundwater systems.

GEOP007

Feasibility studies with GeoPET: microbial processes tomography in geological environments

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Positron emission tomography (PET) is widely used in radiology to visualized cancer cells and infections in human tissue. This functional imaging technique is commonly used in order to visualize processes in the body with the help of radioactive substances. In addition, this method has been used for the investigation of transport processes in geological materials [1].
The aim of our work is the development of a method for the application of PET for geo-microbial research. We will use this technique for the spatio-temporal visualization of the mobility of bacteria in geological matrices. The visualization of the biofilm formation and their influence on flow paths in different matrices is an important aspect as well. In addition, it offers the possibility to get insights into microbial processes (e.g. bioleaching) in opaque materials.
Some basic investigations for the experimental set-up are necessary. First, the method requires an efficient non-toxic method that allows the selective labeling of bacteria without interfering with the geological matrix. Currently, we are investigating a selection of antimicrobial peptides for their use as appropriate marker. Second, appropriate bacteria are selected for further investigation. *Pseudomonas fluorescens* and *Lysinibacillus sphaericus* JG-A12 were chosen as typical organisms living in soil. Third, the interactions of the different compounds and the influence of experimental parameters need to be tested. For further experiments it is very important to examine the stability of the label as well as adsorption and desorption processes of the PET nuclides and of the labeled substances in conjunction with different matrices. The characterization of model columns that are filled with quartz gravel or copper ore needs to be investigated as well. Fluid dynamics and structural parameters like porosity and the internal surface area have to be investigated.
In this study we present most recent results on the development of a PET-method that allows the in-situ labeling of bacteria and the visualization of their mobility in geological matrices.

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GEOP008

Structure and function of microorganisms in the methanic zone of the Helgoland mud area, North Sea

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In many marine sediments, sulfate and methane depletion are coupled giving rise to a sulfate-methane transition zone (SMTZ). Sulfide might diffuse downward from the SMTZ into the methanic zone and react abiotically with Fe (III) minerals buried over geological time scale. This results in the formation of Fe (II) and elemental sulfur which can now be disproportionated into sulfate and sulfide, thereby, fuelling a cryptic sulfur cycle (1). In our samples from the Helgoland mud area, only negligible amounts of sulfate and sulfide was detected beyond 50 cm, whereas Mn^{2+} and Fe^{2+} in pore water still occurred down to 500 cm below the seas floor at concentrations of up to 100 μM and 350 μM respectively. This leaves open the possibility of a microbe-mediated iron (III) reduction. To reveal the possible microbial involvement of iron (III) reduction in the methane zone, Bacteria and Archaea community present therein was characterized by culture-independent molecular analysis. A 500 cm sediment core spanning the SMTZ down to the methane zone was obtained from the Helgoland mud area using a gravity corer (GC) while a 40 cm core was obtained with a multiple corer (MUC). The gravity core was sectioned in 25 cm interval down the depth while only the top 10 cm (zone of Mn^{4+} and Fe^{3+} reduction) of the MUC was sampled in 5 cm intervals. DNA was extracted from sediments of all depths sectioned and subjected to Terminal Restriction Fragment Length Polymorphism (TRFLP), qPCR and 16S rRNA-, *mcrA*- and *dsrB*-targeted 454 pyrosequencing. Bacteria and Archaea TRFLP profiles obtained from the SMTZ down the core were similar but different from surface sediment profiles. qPCR data based on 16S rRNA gene also revealed that in the Helgoland mud area, Bacteria counts ranged from 10^8 - 10^9 gene copies/g wet sediment while Archaea counts ranged from 10^7 - 10^8 gene copies/g wet sediment throughout the depths. Comprehensive analysis of 454 pyrosequencing data is currently underway.

Reference(s):
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GEOP009

Methanogens at the top of the world

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Methanogenesis is the last step in the anaerobic biodegradation of organic matter. The process occurs primarily in permanent anoxic environments, but in recent studies it has been shown that it is not restricted to these anoxic environments only but can also be found in many aerated, upland soils, including desert soils (Angel et al. 2012). The soil in deserts is typically covered by a unique surface layer termed biological soil crust (biocrust). This layer is a few millimetres thick, densely colonized by microorganisms and exhibits most microbial activity.

The objective of this study was to investigate whether methane production can also be found in high-altitude cold deserts in the Himalayas (Ladakh, India). For this purpose, gas measurements, stable isotope analysis and molecular fingerprinting were employed. Soil samples from three different vegetation zones (semi-desert, steppe and subnival zones) as well as from frontal and lateral moraines of receding glaciers were incubated at 25 °C under anoxic wet conditions to induce methanogenesis.

Using these methods we could show that all tested sites were methanogenic under anoxic conditions, although young moraine soils were only partially active. Soils covered with biocrust showed higher methane production rate compared to their barren counterparts, similar to the findings from hot deserts. The measured isotopic signal indicated that methane was produced from both hydrogenotrophic and aceticlastic methanogenesis. Analysing the community using T-RFLP fingerprinting it could be shown that the methanogens were of the families *Methanosarcina, Methanocella* and possibly also *Methanobacterium*. These methanogens became enriched after incubation.

Altogether, our results demonstrate the existence of an active methanogenic community even in such extreme aerated and cold soil environment. Moreover, methanogens were already present in the early stages of moraine soil development at high altitudes, demonstrating that they are an integral part of early successional processes.

Angel R, Claus P & Conrad R (2012) Methanogenic archaea are globally ubiquitous in aerated soils and become active under wet anoxic conditions. *ISME J* 6: 847-862.

GEOP010

Biogeochemical analysis as a tool to isolate extremophilic Archaea

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We studied a microbial snottite biofilm in an abandoned pyrite mine in the Harz mountains. The organisms that build up the biofilm grow at pH about 2.3 and sulfate concentrations of up to 200 mM. They are dependent on the oxidative dissolution of pyrite as the primary energy and on CO_2 as the primary carbon source. The microbial community is mostly composed of bacteria, predominantly by the genera *Leptospirillum* and *Acidithiobacillus* (Ziegler et al. 2012). Organisms belonging to both genera thrive in the vicinity of the biofilm. Surprisingly, the respiratory activity of aerobic organisms lead to a large anoxic zone beginning roughly 700 μm away from the outer surface. In this inner anoxic area Archaea are present and comprise a large part if not even the majority of the community. Recent metagenome analysis of the snottite biofilm revealed that deep branching Euryarchaeota and species closely related to so far uncultured ARMAN ("Archeal Richmond mine acidophilic Nanoorganisms", J. Baker et al. 2006) build the archaeal community. Based on our oxygen, CO_2 and pH analyses we designed a medium for enrichment cultures which included 20 mM ferric iron and casein. Hydrogen and carbon dioxide were supplied in the gas phase. Using CARD-FISH and PCR of the 16S rDNA we could show an enrichment of so far uncultured Achaea from the class *Thermoplasmatales*. After prolonged incubation time, even ARMAN could be detected and transferred several times. Surprisingly, the enrichment was not possible until autoclaved biofilm material was added as an additive to our cultures.

At the moment we focus on establishing pure cultures of the enriched *Thermoplasmatales* and we further try to isolate the growth supporting substance of the biofilm.

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Ziegler, S., Dolch, K., Asskamp, M., Eusterhues, K., Kriews, M., Wilhelms-Dick, D., Göttlicher, J., Majzlan, J., and Gescher, J. Oxygen dependent niche formation in an acidophilic consortium built by Archaea and Bacteria. Submitted to *ISME Journal*.

GEOP011

Multiplex quantification of pathogens and indicator organisms in water using the MCR 3 analysis platform for DNA microarray read-out

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We report on a new method for the rapid and multiplexed analysis of pathogens and indicator organisms in water samples. The research concerning the monitoring of the microbiological water quality and in particular the rapid and parallel detection of pathogenic microorganisms and viruses remain great challenges in environmental biotechnology, ecotoxicology, and public health maintenance. The quantification of waterborne pathogens at low concentrations demands rapid and efficient concentration methods which are compatible to cell cultivation assays or bioanalytical detection methods. At least 10 L of water have to be concentrated to 1 mL to analyse microorganisms and viruses in water by qPCR or DNA microarrays. This was manageable by combining crossflow ultrafiltration with monolithic affinity filtration.[1] Bacteriophages MS2 were spiked in tap water and concentrated with the new CUF-MAF concentration method by a volumetric factor of 10^4 within 33 min. Furthermore, the detection limit for quantification of bacteriophage MS2 by quantitative reverse transcriptase PCR (qRT-PCR) could be improved from 79.47 to 0.0056 GU/mL by a factor of 1.4×10^4. We have built up an automated instrumentation (MMC 3) for the combined concentration process which is characterized with different water samples (tap water, ground water, surface water, and treated waste water). For the multiplexed quantification of microorganisms and viruses in water matrices DNA microarrays after stopped PCR amplification and single strand separation[2] were analysed with the automated chemiluminescence flow-through microarray analysis platform MCR 3. The readout system was equipped with a temperature regulation system for the DNA microarray chip loading unit to increase the efficiency of DNA hybridization. The DNA probes for viruses (norovirus, adenovirus, bacteriophages MS2 and PhiX174) and bacteria (*E.coli*, *E. faecalis*, *Legionella* sp, *P. aeruginosa*) were spotted on a chemically modified glass surface. The parallel quantification was possible in 30 min with detections limits down to 1.5×10^2 GU/mL.

1 Pei, L., Rieger, M., Lengger, S., Ott, S., Zawadsky, C., Hartmann, N.M., Selinka, H.C., Tiehm, A., Niessner, R., Seidel, M., *Environ. Sci. Tech.* **2012**, 46, 10073-10080.
2 Donhauser, S.C.; Niessner, R.; Seidel, M., *Anal.Chem.* **2011**, 83, 3153-3560.

GEOP012

Mechanisms and microorganisms involved in degradation of BTEX and PAH under methanogenic conditions

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For over 80 years there is increasing evidence of active microorganisms in petroleum reservoirs (Bastin et al., 1926;). But only in 1999 the first methanogenic cultures were described degrading hydrocarbons under anoxic conditions to methane (Zengler et al., 1999).

To obtain more insights in the major players involved in methanogenic hydrocarbon degradation, enrichments from different habitats were studied for their capability to degrade alkanes and aromatic compounds. To answer the question of who is doing what in the degradation of these complex hydrocarbons, culture dependent and independent techniques are applied. A detailed analysis of the microbial community in these different enrichments from e.g. shallow and deep subsurface terrestrial and marine systems, resulted in a relatively similar composition, independent of the sampling site. Methanogenic archaea, members of the *Syntrophaceae* and sulfate-reducing prokaryotes contributed to the community.Ongoing work focuses on more complex aromatic compounds, like BTEX and polycyclic aromatic hydrocarbons. Several methanogenic enrichments on e.g. 2-methyl-naphtalene, naphthalene, ethylbenzene and toluene have been obtained, and the conversion of hydrocarbons to methane was confirmed by ^{13}C-labeling. To explore the mechanisms involved in hydrocarbon degradation the enrichments were analysed for the presence of the functional genes *bamA* and *bssA*, encoding for important enzymes involved in the degradation of aromatic compounds. The results indicated a broad phylogenetic range of hydrocarbon degraders present, with differences between cultures. Using 13C-labeled substrates combined with SIP of proteins and DNA, the actively involved microorganisms could be identified. Labeled proteins belonged to methanogens, SRB and Syntrophus species, thus confirming the gene-based analyses. In addition the results from the enrichments are compared with data from environmental samples, e.g. from oil coal and gas reservoirs, to determine the *in situ* importance of the enriched hydrocarbon degraders.

Bastin ES,Greer FE,Merritt CA,Moulton G. The presence of sulphate reducing bacteria in oil field waters.Science.1926 Jan 1;63(1618):21-4.
Zengler, K., Richnow, H. H., Rossello-Mora, R., Michaelis, W. & Widdel, F. Methane formation from long-chain alkanes by anaerobic microorganisms. Nature 401, 266-269 (1999).

GEOP013
Interactions of bacteria with minerals with respect to physical surface properties

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In soils, microorganisms interact closely with minerals. The particles form the habitat for the microbes, while mineral surfaces are affected by several processes as well. These interactions have far-reaching consequences for soil properties and functions. Recent studies have shown that microbial biomass residues contribute to a large extent to the formation of soil organic matter. In particular, cell envelope fragments have been observed to be stabilized in soil. Recent studies have also shown that osmotic stress increases the hydrophobicity of bacterial cell surfaces. If microorganisms are an important source of soil organic matter, the attachment of cells and their residues should affect soil interfacial physical properties. This could explain the observed hydrophobization of soil organic matter after drought periods.
Here, we will present the results of experiments, where pure cultures of *Pseudomonas putida* as a model bacterium were exposed to osmotic stress and mixed with several types of quartz sand. We hypothesize that the effect of salt stress on bacterial surface hydrophobicity is reflected in the surface hydrophobicity of cell-mineral complexes. The associations of those complexes were analyzed for wettability and nanoscale thermal and surface properties. The wettability of the organo-mineral model systems were measured by determining the solid-water contact angle on a small scale. Furthermore, the solid surface free energy was calculated for the characterization of the adhesion properties. The thermal and surface properties were characterized by using AFM (atomic force microscopy) and AFM-nTA (nanothermal analysis).
The results of the experiments will provide insights into the interaction of bacteria with minerals and the effect of microorganisms and their residues on the physical surface properties of mineral particles.

GEOP014
Modeling microbial driven nitrogen turnover processes in soil

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Nitrogen turnover is one of the keyholes of biomass evolution, influencing a wide range of topics from biodiversity up to productivity of agricultural crop systems. Our objective to describe the dynamics of nitrification, denitrification, and nitrite respiration in soil in dependence of available nitrogen compounds, and to consider explicitly the activity of the microbial community under different oxygen conditions.
To reach that objective we defined a simulation model in a way that under aerobic conditions mainly nitrification takes place, while it is inhibited under anaerobic conditions. Consequently, the abundance of the nitrifying microorganisms increases and decreases depending on the oxygen conditions. Denitrification and nitrite respiration only occur under anaerobic conditions, aerobic conditions block it. Denitrifying microorganisms respire oxygen when it is available and switch to nitrate, nitrite, and nitrous oxide once it is depleted. The growth of the nitrite respiring microorganisms is inhibited by oxygen.
Using the Monte-Carlo sensitivity analysis technique the model was analyzed to identify the most influential parameters. It turned out in the analyses that the oxygen status of the soil had the largest influence and that the sensitivities of some parameters were different under changing oxygen supply. Simulations studies for nitrogen and biomass dynamics for different oxygen states in soil are shown that are compared to experimental data. Preliminary experiments with two different soils have been carried out. The samples were adjusted to two different water contents to simulate aerobic and anaerobic conditions. After addition of ammonium and nitrate ammonium, nitrite, and nitrate concentrations and the potential denitrification rate over time were measured. The experimental results are in good accordance with the simulation. Further experiments are planned to calibrate and validate the model.

GEOP015
Investigation of the degradation of 13C-labeled fungal biomass in soil - carbon balance in a soil bioreactor system

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Nutrient balances and degradation processes in boreal forests are mainly influenced by interactions of plant roots and ectomycorrhizal fungi. Plants benefit from nitrogen compounds provided by their symbiotic interaction partner. In return ectomycorrhiza are provided with large amounts of carbon from the plants which is used for the synthesis of hyphal networks in soil and for metabolic activity for nutrient uptake.
Therefore ectomycorrhizal fungi are an important sink for carbon in most boreal forest ecosystems. Their hyphae constitute a major part of soil biomass and, ultimately, a major source for soil organic matter (SOM) formation. While plant-fungal nutrient exchange has been analyzed extensively, the experiment is focused on the fungal contribution to SOM formation.
The fate of the ectomycorrhizal biomass in soil is investigated in a soil bioreactor system. 13C-labeled fungal biomass is incubated in a podzol from a typical forest site over a long period of time (8 months). The results of this experiment will give hints for degradation processes in soil. The experiment aims to balance C turnover processes by the extraction of living and native biomass and comparing their isotopic signatures to total SOM constituents. Furthermore typical biogenic compounds like lipids, amino acids and amino sugars will be extracted to reveal degradation processes.
On the one hand the setup of the long term incubation experiment will be presented. On the other hand the carbon balance will be shown as first results.

GEOP016

Biotic an abiotic iron oxidation at circumneutral pH are linked, inseparable, processes over short and long time scales

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The oxidation of Fe^{2+} can occur through biotic and abiotic processes. Chemical oxidation of Fe^{2+} is a function of pH and O_2 concentration. Biological oxidation of iron is carried out by a diverse group of autotrophic and heterotrophic bacteria. These bacteria use O_2 or NO_3 as terminal electron acceptors allowing them to oxidize Fe^{2+} either aerobically or anaerobically. At circumneutral pH biotic and abiotic Fe oxidation occur at similar rates competing one with the other. We used recent iron oxidizing mats to assess the potential microbial impact on the deposition of banded iron formations (BIF). Iron oxidizing microbial mats, visually dominated by *Gallionella* -like stalks were collected in a time series of long term experiments connected to groundwater aquifers in the Aspo Hard Rock Laboratory (Sweden). Rare Earth Elements (REE) analysis in mats of different ages and in parallel abiotic precipitation experiments. showed similar enrichment patterns as BIF implying that microbial redox conversion of Fe and REE does not significantly affect the REE pattern. Enrichment of Mg, Ca, Na and K due to gypsum precipitation was observed only in aged microbial mats. This is attributed to CO_2 uptake by autotrophs and subsequent changes in the carbonate system. Therefore, secondary precipitation of minerals is an indication of microbial activity but provides no direct information regarding its contribution to Fe^{2+} oxidation. To separate between biotic and abiotic iron oxidation we conducted a series of incubations using live and killed environmental samples spiked with $^{57}Fe^{2+}$. Solid phase and water were separately analyzed to asses Fe oxidation and reduction rates respectively. No significant difference was found between the rates in live and killed samples. This suggests that in natural systems it remains rather difficult to separate biotic from abiotic iron oxidation. Biotically oxidized Fe^{2+}, will further serve as a nucleation matrix for abiotic precipitation of Fe while at the same time both are susceptible to iron reduction. The latter, though mainly a biotic process, can be mediated abiotically as well via reduced organic compounds such as humic acids. To conclude, biotic and abiotic Fe cycling occur in natural systems, however, separating one from the other is a theoretical issue not feasible at present state of knowledge.

GEOP017

Investigating bacterial community associated with iron oxide clogging in technical wells

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Iron ochre incrustations pose a challenge for the functioning of groundwater wells. Microbes play a major role in iron oxide formation, either through oxidizing ferrous iron for electron generation, or through changing the local environment or simply depositing iron minerals due to passive adsorption. A deeper understanding of iron ochre formation is necessary to develop strategies against well clogging. The goals of this project include 1) to reveal the phylogenetic diversity of microbes associated with the ochres in mining wells; 2) to quantify iron cycle related bacteria in ochres, and 3) to link the environmental factors to the bacterial community structure and iron mineral formation.
We have investigated 16 wells with a pH range 4.5 to 7.5 located in an open-cast lignite mine in eastern Germany. The groundwater has high concentrations of Fe(II) (0.5~7 mM) and DOC (1.43~12.59 mgL^{-1}). Pyrosequencing data showed that the phylum Proteobacteria represented the most abundant group (63.75% of the total sequences), followed by Chloroflexi(8.52%) and Actinobacteria(6.36%). Chlorobi, the green sulfur bacteria, ranged from 0.53% to 4.72% of the total bacteria similar to *Acidobacteria*. Iron reducers, including *Geothrix*, *Rhodoferax*, *Geobacter* were detected at low relative abundance (Gallionella were the most dominant group in ochres. 16S rRNA gene copies of *Gallionella* detected by qPCR covered 50% to 90% of total bacteria, in accordance to the result of pyrosequencing analysis. Acidophilic iron oxidizing group *Ferrovum* (0.04~5.39%) and *Acidomicrobium* (0.17~2.35%) were also detected by qPCR. The relative abundance of iron oxidizing bacteria were positively correlated to Fe(II) and oxygen content in well water, negatively influenced by well water pH, while not affected by DOC. Raman spectroscopy showed that ferrihydrite was common in wells with circumneutal pH while schwertmannite dominated the ochres in the slightly acidic wells. The natural microbial iron potential was rather low but could be increased by addition of extra carbon sources. Gradient tubes used to enumerate and isolate iron-oxidizing bacteria retrieved up to 10^6 gradient favoring iron-oxidizing bacteria in all ochre samples. The isolated organisms were related either to autotrophic iron oxidizer *Siderooxydans lithotrophicus*(97% similarity) or to *Thiomonas* sp. NO115(99% similarity). In conclusion, the microbial communities of ochres in wells were dominated by iron bacteria, particularly iron oxidizers *Gallionella*, which might have initiated the formation of ochres.

GEOP018

Microbial abundances and enzyme activities in soils along the Franz Josef chronosequence, New Zealand

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Soil chronosequences provide a unique opportunity to study soil formation in relation to microbial colonization and activity over time. Changing nutrient concentrations in soils of different ages likely affect the number of microorganisms and the activity of extracellular enzymes. The main objective of this study was to test the effect of nutrient and organic matter availability over whole soil profiles on the abundance and activity of the microbial communities with emphasis on soil formation. We focused on microbiological processes involved in nitrogen and phosphorus cycling at seven sites along the 120,000-year Franz Josef soil chronosequence under temperate rainforest. Microbial abundances (microbial biomass and total cell counts) and enzyme activities (protease, urease, aminopeptidase, and phosphatase) were determined in complete soil profiles and related to nutrient contents and pedogenetic soil properties. Both, microbial abundances and enzyme activities decreased with soil depth at all sites. In the organic layers, microbial biomass and the activities of N-hydrolyzing enzymes showed their maximum at the intermediate-aged sites, corresponding to a high aboveground biomass. In contrast, the phosphatase activity increased with site age. The activities of N-hydrolyzing enzymes were positively correlated with total carbon and nitrogen contents, whereas the phosphatase activity was negatively correlated with the phosphorus content. In the mineral soil, the enzyme activities were generally low and decreased with depth; thus reflecting the limited nutrient availability caused by low contents of organic matter and presence of P-fixing minerals such as hydrous Fe oxides. However, cell-normalized activities of N-hydrolyzing enzymes in the mineral soil were significantly higher than in organic layers, indicating a much larger investment of subsoil microorganisms to meet their nutrient demands. Our data, therefore, suggest a strong pedogenetic influence on nutrient cycling in topsoil versus subsoil environments. To determine whether these pedogenetic parameters alter the microbial diversity the community composition is under investigation by 454 pyrosequencing and real-time PCR of 16S rRNA and functional genes involved in nitrogen cycling.

GEOP019

Reductive dehalogenation of brominated aromatics by anaerobic bacterial strains

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Brominated aromatics are widely used as flame retardants, fumigants, agrochemicals, pharmaceuticals, or herbicides in industry and daily life in many parts of the world. Now, brominated aromatics are ubiquitous in the environment, and these compounds threat human and ecosystems health. Because of the chemical structures and properties many brominated aromatic compounds are resistant to chemical and microbial attack under natural conditions. Thus, here we investigate microbial processes transforming brominated aromatics to nontoxic compounds.
Anaerobic reductive dehalogenation is commonly used for eliminating persistent chlorinated compounds in natural and engineered bioremediation efforts. In our group brominated benzenes have already been proved to be fully debrominated by *Dehalococcoides* sp. strain CBDB1[1]. Now, several other mixed and pure cultures belonging to the Fimicutes were also found to be able to debrominate brominated benzenes with different specificities. We applied the different strains to brominated flame retardants to investigate debromination of more complex brominated contaminants. Bromide release,

concentration changes of substrates, and cell growth were monitored to identify debromination activity and to correlate with microbial growth. Further, we will study the compound-specific carbon stable isotope fractionation of debromination reactions to obtain initial indications about the enzyme mechanism and to compare with transformation of chlorinated compounds.
Acknowledgement: This work is supported by the Chinese Scholarship Council (to CY), the VIED, Vietnam (to THD) and DFG FOR1530 (to LA).

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GEOP020
Microbial community shift under simulated climate warming in a permafrost soil of Herschel Island, Northwest Canada

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Permafrost ecosystems harbor large amounts of organic carbon due to low annual average temperatures and low microbial decomposition rates. Increase of temperature due to climate change is predicted to take place faster in the Arctic than in any other region. Therefore permafrost soils play an important role in methane and carbon cycle. Raising temperatures can increase microbial turnover and carbon release (CO_2, CH_4) which could reinforce climate change through a positive feedback loop. Very little is known about the reactions of microorganisms of these habitats to environmental changes. The aim of this study was to gain insights into the effects of raising temperatures on the diversity and abundance of microbial communities of permafrost soils, especially of those involved in the methane cycle. For this purpose an incubation experiment was conducted with intact soil cores from the active layer of a permafrost soil of Herschel Island, Canada. The soil cores were exposed in a permafrost microcosm [1] to increasing temperatures from 10°C (in situ summer temperature) up to 30°C on the surface and from 0°C to 14°C at the permafrost table within 8 months. Soil sampling for molecular analysis was done each 8 cm every two months was followed TRFLP fingerprint analysis based on 16S rRNA genes and qPCR based on the functional genes pmoA and mcrA. Clone library analysis are in progress to complete the information obtained from the fingerprint approach.

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GEOP021
To evaluate the *in situ* degradation of brominated compounds by stable isotope and molecular biological analysis

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Brominated organic compounds (BOCs) have a broad spectrum of applications in the form of flame retardants, pharmaceuticals or herbicides and therefore are produced in an increasing amount per year with the United States, Israel and China as the main producers. Many of these compounds are considered to have toxic, carcinogenic or mutagenic effects. Additionally, brominated compounds are widespread contaminants in sediments and water. Concepts to quantify the *in situ* degradation of BOCs (mainly in the form of dehalogenation) in the anoxic environment are lacking and degradation pathways and reaction mechanisms have not yet been fully elucidated.
We therefore investigated whether compound-specific isotope analysis (CSIA) in combination with molecular biological approaches could be used to assess the *in situ* anaerobic biodegradation of brominated compounds. Laboratory reference experiments showed that a significant enrichment of ^{13}C in the added substrate occurred during microbial dehalogenation of brominated ethenes, however, patterns were different from parallel experiments with chlorinated ethenes. These results suggest the potential use of CSIA for *in situ* assessments of reductive debromination, however, further research is warranted for an understanding of the observed differences. To further explore this potential, a field site in Israel, where brominated as well as chlorinated ethenes and benzenes are found in high concentrations, was investigated and the isotope patterns of these compounds were analyzed. Preliminary results revealed an *in situ* degradation of vinyl bromide whereas vinyl chloride seems to be a product from the dechlorination of higher chlorinated ethenes (e.g. tri-/dichloroethene), shown by the change of the ^{13}C isotope signature from enriched to depleted. In contrast, the isotope values of monochlorobenzene as well as brominated toluene did not change along a predicted groundwater flow, suggesting that these compounds were not degraded. Using taxon specific PCR of organohalide respiring microorganisms, *Dehalococcoides*-like bacteria were detected in all samples, whereas *Geobacter*-like bacteria were detected only selectively. Further analysis of the overall diversity as well as detection of the responsible genes (functional marker genes) will contribute to assess whether dehalogenation is taking place and which organisms are involved in this process.

GEOP022
Characterization of indigenous oil field microorganisms for microbial enhanced oil recovery

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Due to rising energy and oil demand as well as a decreasing production of mature oil reservoirs microbial activities and metabolites also became a focus of attention for enhanced oil recovery (MEOR, microbial enhanced oil recovery). The objectives of this study were (1) to characterize the indigenous microbial community in a German oil field, (2) to investigate the dependency of microbial activity/diversity on different sampling strategies and (3) to study the influence of the incubation pressure on bacterial growth and metabolite production. Fluids were sampled at the well head (surface) and *in situ* at approx. 700 m depth to collect uncontaminated production water directly from the field and under reservoir pressure of 30.8 bar (subsurface). In the lab the pressure was either released quickly or slowly to assess the sensitivity of microorganisms to rapid pressure changes. Quantitative PCR resulted in higher microbial cell numbers in the subsurface sample than in the surface sample. Biogenic CO_2 and CH_4 formation rates were determined under atmospheric and high pressure conditions in the original fluids with highest rates in the surface fluid. Interestingly, no methane was formed in the pure fluid samples. While nitrate reduction was exclusively detected in the surface fluid, sulfide formation also occurred in both subsurface fluids. Increased CO_2 formation was measured for a variety of substrates in the surface fluids, while only fructose and glucose showed an effect for the depressurized subsurface sample. Stable enrichment cultures were obtained in complex medium under atmospheric and *in situ* pressure that originated from the depressurized subsurface fluid. Growth experiments with constant and changing pressure and subsequent DGGE analysis of bacterial 16S rRNA genes revealed that the pressure treatment did not affect the bacterial community composition. Our results indicate that bacteria in the enrichment culture can tolerate pressure changes between atmospheric and *in situ* reservoir pressure. Since substantial differences were observed between the surface and subsurface fluids in terms of microbial activity, the selection of the sampling strategy should further be considered for MEOR research and industrial application.

GEOP023
Interaction between *Beggiatoa* and cyanobacteria in biofilms from sulfidic springs

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We used microsensors to study the interaction between *Beggiatoa* and cyanobacteria in biofilms covering sediments and rocks in sulfidic springs that emerge from the Frasassi cave system, Italy. Depending on the availability of oxygen, light and hydrogen sulphide, the biofilm structure varies between two end-members. Biofilms of type 1, which form when the spring water is microoxic (O_2 < 10 μM), are characterized by a cyanobacterial layer on top of a thin white layer, in which *Beggiatoa* are abundant. Photosynthetic activity of the cyanobacteria shifts between anoxygenic and oxygenic, depending on sulphide concentration and incident light intensity. The *Beggiatoa* clearly benefit from the oxygenic photosynthesis by positioning themselves underneath the cyanobacterial layer and profiting from the excessive oxygen fluxes from above and sulphide fluxes from below. In biofilms of type 2, which form in oxygenated

spring waters (O_2 > 80 μM), a thick white layer of *Beggiatoa* filaments covers a thin cyanobacterial layer. The *Beggiatoa* can use the oxygen supplied from the water column for sulphide oxidation independent of light and oxygenic photosynthetic activity over a complete diurnal cycle. By reflecting most of the incident light, the thick layer of white *Beggiatoa* filaments limits phototrophic activity to anoxygenic photosynthesis in the cyanobacterial layer underneath. We conclude that in type 1 biofilms not only phototrophic, but also chemolithotrophic activity by *Beggiatoa* indirectly depends on light as the energy source, while in biofilms of type 2 *Beggiatoa* have a continuous light-independent access to their energy sources and therefore outcompete the cyanobacteria.

GEOP024

Microbial control on P/Fe particle formation

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As a transition zone between water column and sediments, the benthic boundary layer (BBL) plays a key role in the regeneration of biolimiting nutrients. In particular, the fluxes of phosphorus (P) and iron (Fe) from the sediment to the overlying water column are strongly modulated by particle formation in the BBL. The role of microorganisms in the formation of particulate P and Fe in the BBL is poorly understood.
We investigated the role of microbial P uptake and release in the presence of Fe- and P-rich particles. These processes cannot be readily distinguished by using bulk extraction methods and require novel high resolution imaging approaches. In a first stage, we combined 33P incubations and pure culture experiments with Raman Laser Microscopy and nanoSIMS to to visualize and quantify particulate P as storage products in single cells and as extracellular precipitates. We used model organisms *Alteromonas macleodii* and Candidatus *Brocadia sp*., and subsequently applied the approach to a sediment enrichment culture.
The obtained insight into the microbial Fe and P cycling is vital for our understanding of how primary production in the oceans is controlled.

GEOP026

Elucidation of the distribution and physiological role of *hypX* gene in high affinity H_2-oxidizing bacteria

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Hydrogenases (H_2ases) are catalyzing the interconversion of H_2 into protons and electrons. These metalloenzymes are used by microorganisms to generate energy or control the redox potential in the cells. Intensive efforts are currently invested to integrate H_2ases or synthetic material mimicking the active site of these enzymes into biofuel cells or biohydrogen production processes. One key limitation of these applications is the inactivation of H_2ases upon O_2 exposure. *Actinobacteria* possess structural (*hhySL*) and auxiliary (*hypABCDEFX*) genes encoding a new type of [NiFe]-H_2ase. This novel enzyme is unusually O_2-tolerant, while showing high affinity to H_2. *Streptomyces* representatives are displaying the highest affinity to H_2 and only the bacteria belonging to this genus possess the H_2ase auxiliary gene *hypX* - for which the function is unclear. The objective of this research is to assess the distribution of *hypX* in H_2-oxidizing bacteria and verify whether *hypX* influences the kinetic properties of [NiFe]-H_2ase. This challenge has been addressed by using physiological and genetic approaches. Until now, phylogenetic analysis unveiled the absence of coevolution between *hypX* and the other genes specifying the structural subunits and the maturation apparatus of high affinity H_2ase. The gene *hypX* is unevenly distributed in *Streptomyces* spp. and its presence or absence does not reflect the kinetic parameters governing H_2 oxidation activity. We showed that *hypX* is expressed as monocistronic mRNA and work is underway to inactivate the gene in the model high affinity H_2-oxidizing bacterium *S. avermitilis*. This mutant strain will show whether *hypX* influences the H_2 oxidation activity or not in *S. avermitilis*.

GEOP027

Basanite vs. copper slag: rock chemistry shapes bacterial communities in epilithic biofilms

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Within the scope of an environmental impact assessment of industrially produced copper slag, which is to be used as armor stone in waterways, we investigated how different rock materials may affect the structure and diversity of bacterial biofilm communities. Rock chemistry can take effect in two ways: i) by direct contact and/or ii) by leached compounds. The experiments were performed in six indoor stream mesocosms filled with water and sediment of the river Rhine and the test material. Next to copper slag, basanite was tested as a reference armor stone of natural origin. The two test materials differed in mineral composition and in type and amount of metal(loid)s leached into the aquatic environment. In a first experiment, test materials were applied as crushed sand (grain size

GEOP028

Important partners in subaerial biofilms: melanised microcolonial fungi as climate indicators and instruments of material deterioration

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Every material exposed to the atmospheric environment can be covered by subaerial biofilms (SAB´s). The appearance of SAB´s can differ depending on climatic conditions, the nature of substrate and the duration of the material exposure to the atmosphere. However the inherent "core" of SAB settlers consists of phototrophic algae, cyanobacteria, heterotrophic bacteria and melanised microcolonial fungi (MCF). Especially MCF are characterized by their stress-protective morphologies which include a peculiar compact colonial structure, protective cell walls, high melanization and meristematic growth. Because of their ability to exert chemical and physical substrate deterioration and survive under extreme environmental conditions they are of big importance for the SAB formation, material alteration and are pioneer settlers on diverse air-exposed surfaces. By repeated investigation of the seasonal MCF diversity of northwest and southeast exposed facades we could show that they are also indicators for microclimatic conditions.
SAB´s are of big importance for the building industry because they are not only present on all atmosphere exposed materials, but actively participate in mineral weathering and material deterioration. Recently we described SAB on photovoltaic panels and could isolate cyanobacteria and MCF from these glass surfaces. The SAB mediated deterioration of the glass surface together with absorption and scattering processes of SAB organisms have a considerable impact on the efficiency of solar plants.
With the help of laboratory model biofilms we are able to investigate biofilm-material interactions as well as SAB caused material deterioration. A physiological characterization and genetic manipulation of the model biofilm partners will help to better understand the formation of SAB´s on various materials. Recently we were able to establish a transformation protocol for *Knufia petricola* A95, a fast growing MCF and appropriate partner of a model biofilm in material sciences.

GOMV001

Less is more - Bacterial gene loss results in a division of metabolic labour and the formation of intercellular networks

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Cross-feeding interactions, in which bacterial cells exchange metabolites to the benefit of the interacting partners, are very common in the microbial world and have been shown to readily evolve under laboratory conditions. This type of ecological interactions, however, is inherently difficult to study within natural microbial communities, because of the complexity to identify the chemical nature of the interaction as well as difficulties to determine the underlying genetic basis. To solve this problem, we engineered synthetic cross-feeding interactions between two genotypes of *Escherichia coli*. The design of these interactions was guided by prior knowledge on essential features of the focal interactions as well as computational analyses to identify genetic targets. By simply deleting two metabolic genes, we generated a range of genotypes that reciprocally exchanged essential amino acids when cocultured. Surprisingly, in a vast majority of cases, cocultures of two of these cross-feeding strains showed an increased Darwinian fitness relative to unmutated wild type (WT) cells - even in direct competition. This unexpected growth advantage was due to a division of metabolic labour among cooperating cells: the fitness cost of overproducing certain amino acids was less than the energetic gain of not having to produce others when they were provided by their partner. Interestingly, in spatially structured environments (i.e. agar plates), in which amino acids are distributed more locally, cross-feeding consortia could persist and even outcompete non-cooperating types (i.e. WT or auxtrophs), while in a spatially unstructured environment (i.e. liquid culture), cross-feeding was the least fit strategy. This result emerged both in theoretical, individual-based models as well as in coculture experiments. Our finding provides an adaptive explanation for the ease, with which bacteria enter into metabolic mutualisms with other micro- or macroorganisms and suggest bacteria most likely function as a network of interacting cells, rather than as physiologically autonomous units.

GOMV002

Functional microbial ecology in the gut ecosystem revealed by metaproteomics - from feces to the mucus layer

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The digestion of many food ingredients depends on the action of the gut microbiota which has a significant influence on the health of the host organism. While the phylogenic assessment provides the genetic blueprint, it lacks the proof of functionality. With the development of metaproteomics, the actual functional proteins and their phylogenic affiliation are analyzed. In order to obtain relevant information of gut microbiota, different sample sites from feces to the mucus layer have to be considered. Our group has recently set the methodological pipeline for feces, gut content and mucus layer processing, metaproteome measurement and data evaluation [1-3].
Feces samples from an obese and a lean adolescent were analysed by label-free quantitative metaproteomics. The phylogenetic composition of the proteins revealed a dominance of Bacteroidetes (80%) in the lean sample whereas proteins related to Firmicutes (60%) dominated the obese sample. A functional redundancy in the microbiota of obese and lean humans independent of the phylogenetic composition was shown; however, the evidence collected demonstrates that different minor bacterial taxa seem to be significant active contributors that help provide overlapping and/or complementary functional roles.
The spatial resolution of the microbiota and the functionalities in the different gut sections is mostly unknown. Our data revealed clear phylogenic and functional differences between mucus layer, gut content and feces of rats. The experiments summed 2,802 non-redundant bacterial proteins in total distributed within 24 bacterial phyla. For each gut segment the proteins belonging to Bacteroidetes were always more abundant in the content than in the mucus, whereas for proteins from Firmicutes this was reversed. From proximal to distal regions of the large intestine especially in the mucus, changes in distribution were observed. The phylogeny of distal colon proteins closely resembled the makeup of feces proteins. Thus, the results showed that the mere analysis of feces samples reflects the functions of the gut microbiota only to a minor extent.

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GOMV003

Lasting impact of acute inflammation on murine intestinal microbiota structure and function

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Chronic inflammatory bowel disease (IBD) is presumably caused by an imbalance of intestinal microorganisms and host immune system, resulting in a dysbiosis. It is currently unclear how acute inflammation impacts on functional interaction between microbiota and host. Furthermore it is not known if and how the microbiota recovers after acute inflammation. In this study, we investigated the microbiota response during and after acute inflammation in a murine colitis model triggered by dextran sodium sulfate (DSS).
Metatranscriptomics and 16S rRNA gene amplicon sequencing were employed to determine microbial community structure and function before (day 1) and at the end of DSS treatment (d5), during (d8) and after inflammation (d14, d25).
Shifts in community profiles were already observed at day 5 without apparent changes in host markers of inflammation and were most pronounced during inflammation (d 8) with decreases in the predominant *Clostridiales* and concurrent increase of *Bacteroidales* and the low abundant *Deferribacteriales* and *Enterobacteriales*. Community shifts were caused by shuffling of abundances rather than a replacement of community members. These changes were accompanied by shifts in microbial functions, shown by altered gene expression. *Clostridiales* were heavily impacted as indicated by a 21 and 49% reduction of their major transcripts encoding flagellin subunits, at day 5 and 8, respectively, relative to day 1. The decrease of *Clostridiales* during inflammation correlated with a reduction of transcripts related to butyrate production; the raise of *Bacteroidales* was paralleled by an increase of mucin degradation transcripts. Already at day 14, the microbial community and function shifted back towards a healthy state but did not reach its initial composition. Principal component analysis showed a higher degree of community and function variability after inflammation between the replicates.
In conclusion, the decrease of clostridial flagellins, dominant antigens in murine colitis and IBD, implies a strong reaction of the immune system against selected members of the microbiota. Inflammation impacted host-microbiota interactions by reduction of butyrate synthesis and increased ability for degradation of mucin and lastingly altered the murine microbiota and its function.

GOMV004

Deep sequencing reveals promotion of gastric lactobacilli in weaned pigs by increased dietary calcium-phosphorus levels

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In pigs after weaning, the intestinal microbiota must develop from an immature community into a complex and stable autochthonous microbiota. The supplementation of calcium and phosphorous (CaP) to the diet at levels largely above the requirement is categorized as unfavorable for pig's health but the potential stimulation of beneficial mucosa-associated lactobacilli by dietary CaP as observed in rats [1] could be a promising approach. This study was conducted to investigate effects of different basal diets (wheat-barley or corn) and different CaP levels on mucosa-associated microbial community in stomach, ileum and colon of weaned pigs.
Using a completely randomized design, pigs (n = 32) were fed wheat-barley or corn based diets with two different CaP levels (90% or 170% of the requirement) for 15 days. Genomic DNA isolated from mucosal scrapings of stomach, ileum and colon was used for 16S rRNA gene-targeted pyrosequencing.
Pyrosequencing of 93 samples revealed 4,569 sequences for stomach, 5,191 for ileum and 3,519 for colon on average for each gut site. In total, 12 different bacterial phyla and 418 genera were identified across all samples. *Firmicutes*, *Proteobacteria*, and *Bacteroidetes* were the main phyla. Our data reveal unknown high diversity and species richness in the stomach mucosa. In addition, our results also show that the mucosal microbiome is distinct from the microbiome of digesta. High versus adequate CaP level increased *Lactobacillaceae* by 23% and reduced *Prevotellaceae* by 11% in the stomach (P<0.05). In the ileum, *Enterobacteriaceae* increased by 4% with the high versus adequate Ca-P level (P<0.05). *Lachnospiraceae* increased with the wheat-barley versus the corn diets (P<0.05).
In conclusion, cereals and CaP differently affected the mucosa-associated microbiota at all gut sites. Promotion of mucosa-associated lactobacilli in the stomach by high CaP may be beneficial for gut health of weaned pigs.

[1] TEN BRUGGENCATE et al. (2004)
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GOMV005

Bacterial activation of glucosinolates: Variety is the spice of life

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Glucosinolates (GLS) are secondary plant metabolites in *Brassicaceae*. Some GLS, including glucoraphanin (GRA) from broccoli have been proposed to have chemoprotective properties, while others, such as neoglucobrassicin (NGBS), are possibly genotoxic because their degradation products interact with DNA [1]. Effects of GLS are mainly attributed to their hydrolysis products, the isothiocyanates, which are formed by the plant enzyme myrosinase upon tissue damage. Although myrosinase is largely inactivated by thermal treatment, biological effects of GLS have been observed following the intake of cooked *Brassica* vegetables [2, 3]. Activation of GLS in the gut is most likely performed by intestinal bacteria.
Aim of the current study was to investigate to which extent human gut bacteria contribute to the bioactivation of GLS. Therefore, interindividual differences in bacterial transformation of GLS were studied using faecal suspensions from 20 human donors. Six different GLS from three different GLS classes were investigated. We also tested a plant extract from pak choi sprouts, which contained a mixture of different GLS and other secondary plant compounds. Fermentation experiments were carried out under anoxic conditions for 48 hours at 37 °C. Content of GLS and potential metabolites at different time points were determined using HPLC/DAD.
The bacterial conversion of the six different GLS by the human donors varied widely and was overall low. A quarter of the donors did not activate four of the tested GLS. Only NGBS was activated by all human donors to the corresponding isothiocyanate, which was transformed spontaneously into an alcohol. GRA was primarily reduced to glucoerucin. Bacterial GLS conversion showed no specificity for GLS class. Conversion of GLS in pak choi extract was low compared to the pure substances.
In conclusion, intestinal bacteria possess a myrosinase activity and may therefore be involved in the bioactivation of GLS in the human gut. However, their contribution to GLS transformation *in vivo* is apparently low.

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GOMV007

Intestinal microbiota shifts towards elevated commensal *Escherichia coli* loads abrogate colonization resistance against *Campylobacter jejuni* in mice

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Background: The zoonotic pathogen *Campylobacter jejuni* is a leading cause of bacterial foodborne enterocolitis in humans worldwide. The understanding of immunopathology underlying human campylobacteriosis is hampered by the fact that mice display strong colonization resistance against the pathogen due to their host specific gut microbiota composition.
Methodology/Principal Findings: Since the microbiota composition changes significantly during intestinal inflammation we dissected factors contributing to colonization resistance against *C. jejuni* in murine ileitis, colitis and in infant mice. In contrast to healthy animals *C. jejuni* could stably colonize mice suffering from intestinal inflammation. Strikingly, in mice with *Toxoplasma gondii*-induced acute ileitis, *C. jejuni* disseminated to mesenteric lymphnodes, spleen, liver, kidney, and blood. In infant mice *C. jejuni* infection induced enterocolitis. Mice suffering from intestinal inflammation and *C. jejuni* susceptible infant mice displayed characteristical microbiota shifts dominated by increased numbers of commensal *Escherichia coli*. To further dissect the pivotal role of those distinct microbiota shifts in abrogating colonization resistance, we investigated *C. jejuni* infection in healthy adult mice in which the microbiota was artificially modified by feeding live commensal *E. coli*. Strikingly, in animals harboring supra-physiological intestinal *E. coli* loads, colonization resistance was significantly diminished and *C. jejuni* infection induced enterocolitis mimicking key features of human campylobacteriosis.
Conclusion/Significance: Murine colonization resistance against *C. jejuni* is abrogated by changes in the microbiota composition towards elevated *E. coli* loads during intestinal inflammation as well as in infant mice. Intestinal inflammation and microbiota shifts thus represent potential risk factors for *C. jejuni* infection. Corresponding interplays between *C. jejuni* and microbiota might occur in human campylobacteriosis. Murine models introduced here mimick key features of human campylobacteriosis and allow for further analysis of immunological and molecular mechanisms of *C. jejuni* - host interactions.

GOMV008

Identification and functional expression of isoflavone conversion genes from the human intestinal bacterium *Slackia isoflavoniconvertens*

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Gut bacteria play a crucial role in the metabolism of dietary isoflavones which have been implicated in the prevention of hormone-dependent and age-related diseases. Only the intestinal bacteria are able to catalyze the bioactivation of the soybean isoflavones daidzein and genistein to equol and 5-hydroxy-equol, respectively. Although several equol-forming gut bacteria have been isolated in recent years, the knowledge on the involved enzymes is still scarce. *Slackia isoflavoniconvertens* represents one of the few equol-forming gut bacteria isolated from humans [1]. Growth experiments with *S. isoflavoniconvertens* indicated that the enzymes catalyzing the conversion of daidzein and genistein were inducible by these isoflavones. Using two-dimensional difference gel electrophoresis (2D-DIGE), several proteins were

found to be upregulated in *S. isoflavoniconvertens* cells grown in the presence of daidzein. Based on selected protein sequences, a cluster of eight genes was identified encoding the daidzein-induced proteins. The heterologous expression of three of those proteins in *Escherichia coli* and enzyme activity tests identified them as a daidzein reductase, a dihydrodaidzein reductase and a tetrahydrodaidzein reductase. The combined cell extracts catalyzed the complete conversion of daidzein to equol. The recombinant daidzein reductase also converted genistein to the intermediate dihydrogenistein at higher rates than observed for the conversion of daidzein to dihydrodaidzein. The three reductases were functionally expressed as *Strep*-tag fusion proteins and purified by a one-step affinity chromatography. In addition, the remaining daidzein-induced proteins were successfully expressed in *E. coli* and purified. This provides the basis for the detailed characterization of the enzymes involved in isoflavone bioactivation by *S. isoflavoniconvertens*.

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GOMV009

Survival traits of Enterococcus spp. in the human gut

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The genus Enterococcus represents indigenous members of the human gut microbiota, adapted to the nutrient-enriched, oxygen-depleted and ecologically complex gut environment. However, an increase in the density of colonizing enterococci is a first step for nosocomial enterococcal infection. In this study we investigated the presence of antibiotic resistance genes and virulence factors in Enterococcus isolates from the gut of patients without nosocomial enterococcal infection diagnosed.
Enterococcus spp. isolates were obtained by conventional culture methods from faecal samples of 3 hospitalized patients that received antibiotic prophylaxis therapy, and from ileostomy effluent of 1 subject without antibiotic treatment. All isolates were identified by 16S rRNA gene sequencing and (GTG)-5 PCR for classification of Enterococcus spp. The Minimal Inhibitory Concentration (MIC) of vancomycin was determined by the agar dilution method. In addition, a double diffusion test was performed for macrolide resistance. We investigated the carriage of glycopeptide (vanA, vanB, vanC1, vanC2/C3, vanD, vanE and vanG) and macrolide (ermA,ermB,ermC and mefA/E) resistance genes and 3 virulence-associated genes (esp, asa1 and hemolysin) by PCR.
Of 63 isolates (19 from faecal samples and 44 from ileostomy effluent), 13 (21 %) were identified as E. faecium, 29 (46%) E. faecalis, 5 (8%) E. gallinarum and 16 (25%) E. avium.Only E. gallinarum and E. avium were found in ileostomy samples. A total of 59 isolates (94%) were resistant to vancomycin (MICs > 32 μg/ml), however, only vanC1 gene was found in Enterococcus isolates (E. gallinarum). The phenotype found in all fecal isolates and in 13 (30%) of the ileostomy isolates corresponded to constitutive phenotype (MLSb). We identified ermB genes in 6 isolates from feces and 12 from ileostomy (E. avium and E. gallinarum). All isolates tested showed hemolysin production after incubation in blood agar plates for 24 h. From 63 isolates, 53 (84%) carried the esp gene and 32 (51%) carried an asa1 gene; this gene was present only in E. faecium and E. faecalis isolates.
The study showed a high prevalence of antibiotic resistance genes and virulence factors in Enterococcus isolates from gut microbiota, suggesting that Enterococcus population in the human gut can be reservoirs for antibiotic and virulence genes.

GOMP001

The role of monoamine neuromediators in the microflora-host dialogue

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This contribution deals with recent data available in the literature and the author's own findings concerning the involvement of monoamine neuromediators (MNs) in the chemical interactions between the human host organism and the microflora of the gastro-intestinal tract in health and disease. Two types of data are discussed:
1. The impact of host-released MNs on the microflora. Catecholamines produced in response to stress (including infection) by the human organism significantly stimulate growth, adhesion to mucosa cells, biofilm formation and virulence factor production in a wide variety of enteropathogenic bacteria including enterohemorrhagic *Escherichia coli* strains. They perceive them as analogs of AI-3, a pheromone involved in quorum-sensing communication [1]. In our experiments, MNs including dopamine, norepinephrine, serotonin, and especially histamine stimulated the growth of nonpathogenic microflora exemplified by *E. coli* K-12 and produced effects on the formation of microcolonies in this bacterium [2].
2. The synthesis and release of MNs by symbiotic or pathogenic bacteria. Serotonin, dopamine and norepinephrine, as well as their precursors and oxidative deamination products are contained in microbial cells and released into the culture fluid, particularly at the later culture development stages, as our research with *E. coli* K-12 and the potentially pathogenic food-spoiling bacterium *Bacillus cereus* revealed [3]. Of particular interest is the fact that *E. coli* releases micromolar amounts of DOPA, the catecholamine precursor that crosses the gut-blood and blood-brain barriers.

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GOMP002

Bacterial ATP secretion

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It has been reported that ATP derived from gut commensal bacteria promotes the differentiation of T helper 17 cells in the intestinal lamina propria and it is suggested that the ATP contributes to exacerbation of colitis in mice, however, which bacteria secrete ATP was unknown. Recently, we reported that *Enterococcus gallinarum*, isolated from mice and humans, secretes ATP. In the present study, we aim to investigate the bacterial ATP secretion mechanism.
We examined the ATP-secreting property of 22 enterococcal species, and then we newly identified 7 ATP-secreting enterococcal strains. Among them, *Enterococcus mundtii* secreted the largest amount of ATP, therefore we use this strain for further analyses. To investigate the effective factor(s) for ATP secretion by *E. mundtii*, we used the technique of the omission of medium component, and found that glucose is the most essential for bacterial ATP secretion. Furthermore, *E. mundtii* secreted an equal amount of ATP in aerobic or anaerobic culture conditions, this finding suggested that glycolysis is crucial for ATP secretion. By using energy-deprived cells which were treated with dinitrophenol and completely depleted of intracellular ATP, exponential-phase cells secreted much higher ATP than stationary-phase cells. We examined ATP secretion of 6 strains including *Escherichia coli* and *Staphylococcus aureus* which were reported as non ATP-secreting bacteria, we found that all tested bacteria secreted ATP at exponential phase.
In the presence of glucose, various commensal or pathogenic bacteria such as *S. aureus* secrete ATP in a growth phase-dependent manner, and it is suggested that ATP may involve in bacteria-bacteria communication and bacteria-host interactions.

GOMP003

Establishment of a novel in vitro cell culture model to interfere with *Campylobacter jejuni* infection based on a chicken intestinal enterocyte cell line.

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Campylobacter spec. represent one of the most aggressive zoonotic pathogens worldwide, with even a higher incidence of novel disorders than Salmonella-infections. [1] Campylobacter infections in humans occur due to the consumption of contaminated food, especially with undercooked chicken and raw milk products.
The typical way to interfere in this scenario is the application of antibiotics in the stock breading bearing the hazard of generating unwanted resistance. Until now, there exist no effective alternatives to prevent Campylobacter infections neither in human nor in the primary host, in chicken.

In this regard we established a series of chicken intestinal enterocyte cell lines and generated a novel in vitro infection model to identify possible inhibitors for *Campylobacter jejuni* infections. Based on the fact that different probiotics reduce the adherence from Campylobacter to mucus [2], it was investigated if Bifidobacterium in combination with prebiotics reduces Campylobacter concentrations in chicken *in vivo* [3].
Furthermore, the cell wall of gram-positive probiotics was isolated and its potential to reduce the adherence and invasion of *Campylobacter jejuni* to Caco-2 cells and chicken intestinal enterocytes (8E11 p1/16) was tested. First results indicate enhanced adhesion of C.jejuni 81-176 to Caco-2 and chicken enterocytes as well as higher invasion of chicken enterocytes following to the treatment with cell walls of *Lactobacillus rhamnosus* or *Lactococcus lactis*.

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GOMP004

Implementation of a cellular model for the infection of Salmonella serotype Typhimurium and Enteritidis based on chicken enterocytes: interference with porcine mucin, 3'SL and LNF III.

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Food-borne diseases, mainly submitted by poultry products, are an important public health problem [1]. In order to prevent food-borne illness, the incidence of human pathogens in animal husbandry needs to be reduced and infection studies should be conducted using animal gut cells. Chicken enterocytes cell line developed in the previous study and human Caco-2 cells were used for estimation of Salmonella behavior of the two most commonly serotypes Typhimurium and Enteritidis [2].
The surface of the gastrointestinal tract is covered by mucic glycoproteins, which contain receptors that recognize specific adhesion proteins [3]. In addition sugar molecules present on the cell's surface can act as a 'danger' signals. The danger receptors detect bacterial invasion by binding host glycans exposed on damaged vacuoles [4]. Carbohydrates with high affinity for the cell membrane proteins, are potential candidates for infection inhibitors [5]. Therefore, we investigated the infection of Salmonella serotypes in the presence of porcine mucin, 3'-sialyllactose (3'-SL) and lacto-N-fucopentaose (LNF III).
Our results showed significantly reduced infection of chicken and human enterocytes with S. Typhimurium but not with S. Enteritidis in the presence of porcine mucin. Interestingly, the lowest concentrations of mucin inhibited the infection to a greater extent compared to higher concentrations. The LNF III significantly reduced infection of chicken enterocytes but not of human gut cells caused by serotype Typhimurium. The same trend could be shown also for serotype Enteritidis. The concentration was not essential. The 3'-SL did not reduce infection of any cell type or the infection was even intensified. In conclusion, the porcine mucin and LNF III appear to inhibit Salmonella from interacting with epithelial cell surfaces. The differential outcome of *Salmonella* infection of gut cells suggests differences in the composition of both cell membrane surfaces of the host cells as well of the Salmonella strains.

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GOMP005

Core microbiome of healthy and morphologically altered ileocaecal lymph nodes of slaughter pigs determined by 16S rRNA gene pyrosequencing

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In a previous study we found more than 1/5th of examined slaughter pigs (n>600) showed morphological alterations in ileocaecal lymph nodes, associated with enlargement, purulence, granuloma or cystic formations (unpublished data). The microbial community in lymph nodes of pigs has been studied very rarely until now, mostly using culture dependent methods. The aim of the present study was to identify and characterize microbial community shifts associated with morphological alterations in ileocaecal lymph nodes of slaughter pigs in Austria using 16S rRNA gene-targeted-pyrosequencing. Thirty two healthy, enlarged, purulent and granulomatous (n=8, respectively) lymph nodes were selected from different slaughter pigs and isolated genomic DNA was used for pyrosequencing. The microbial communities of lymph nodes were analyzed using the software mothur. In total ~150,000 reads were clustered in 173 OTUs (distance level=0.03). In healthy and enlarged lymph nodes the most abundant phyla found were *Firmicutes*, *Proteobacteria* and *Bacteroidetes* (>86%). In granulomatous lymph nodes *Tenericutes*, *Actinobacteria* and *Fusobacteria* increased compared to healthy and enlarged lymph nodes ($P<0.05$). In healthy and enlarged lymph nodes analyses on genus level revealed that the most abundant genera were *Haemophilus*, *Bacteroides* and *Acidovorax*-like. Specific shifts of microbial communities could be observed in granulomatous lymph nodes versus healthy lymph nodes, where the abundance of *Pseudomonas, Mycoplasma* and *Propionibacterium* increased ($P<0.05$). In conclusion, ileocaecal lymph nodes harbor an enormous microbial diversity including both non-pathogenic and pathogenic bacterial genera. The knowledge of the microbial core community of ileoceaecal lymph nodes and shifts in pathologically altered ileocaecal lymph nodes helps to facilitate diagnostics in slaughter pigs and to assess potential risk of zoonotic transmission.

GOMP006

Interspecies communication between *Streptococcus mutans* and two other oral pathogens in biofilms

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Virulence traits of *S. mutans,* e.g. genetic competence, biofilm formation and bacteriocin secretion are controlled by quorum sensing. The main signaling molecules involved in this process are the competence stimulating peptide (CSP) and the alternative sigma factor SigX inducing peptide (XIP). Both pheromones induce transcription of SigX, resulting in activation of the late competence genes, including DNA uptake and recombination machinery genes. A *S. mutans* GFP reporter strain for the *sig*X promoter was used to identify oral microorganism influencing genetic competence development of *S. mutans* in dual biofilms. Measurement of GFP fluorescence revealed strong induction of the *sigX* promoter after 8 to 12 h of growth in dual biofilms with *Candida albicans* as well as in dual biofilms with *Aggregatibacter actinomycetemcomitans (A.act.)* but not when the reporter strain was cultivated alone, which was confirmed by microscopial analysis and q-RT-PCR. Filtered supernatants of mixed biofilms activated *sigX* in biofilms of *S. mutans* cultivated alone. However, there was no activation by filtered supernatants originating from single biofilms. Using a whole genome microarray we analysed the transcriptome of *S. mutans* growing with *C. albicans* for 6, 10 and 24 h in mixed biofilms compared to *S. mutans* biofilms growing alone. At 10 h we observed strong induction of the genes encoding XIP (fold change 68), as well as *sigX* (fold change 56) and the downstream late competence genes of the transformasome, suggesting that competence was induced in *S. mutans* in mixed biofilms. Genes regulated by CSP were also activated but at a lower level, e.g. ComCDE, and bacteriocins. Using gene deletion mutants, we analysed the role of two-component systems and pheromone synthesis genes for this interaction. Our data demonstrate for the first time induction of competence in *S. mutans* through interspecies communication with oral pathogens in dual species biofilms.

GOMP007

Use of natural feed additives to control enteric pathogens in organic poultry

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Organic poultry production is one of the fastest growing segments of organic agriculture (20% increase/year in the United States). Despite the fact that most management practices in organic production are designed to promote bird health and prevent disease, the lack of consistently effective organic treatments for enteric diseases can adversely influence the bird's health and the wholesomeness of poultry products. Enteric diseases such as necrotic enteritis and food safety hazards caused by the pathogens Salmonella and Campylobacter, are high priority issues for organic poultry producers. We are evaluating the effects of different management practices (outdoor access, different housing systems and environmental enrichment on the pasture) on the birds, the pasture and on the presence of pathogenic bacteria. In addition, we are evaluating the ability of different natural plant extracts (such as trans-cinnamaldehyde (TC) from cinnamon and eugenol (EG), from clove) to reduce enteric pathogens. For example, to test the ability of TC or EG to reduce Salmonella enterica (SE) colonization, 84 day-old chicks were placed into 6 treatment groups (n=14/group): a negative control (no SE, no TC or EG), EG control (no SE, 1% EG), TC control (no SE, 0.75% TC), a positive control (SE, no TC or EG), an EG challenge group (SE, 1% EG) and a TC challenge group (SE, 0.75% TC). Before the start of each experiment, the flock was screened for any inherent Salmonella. Birds were given ad-libitum access to Salmonella-free feed and water. On d 30, birds were challenged with a four-strain mixture of SE (8 log10 CFU/bird). Two birds from each group were sacrificed after 24 h (d 31) to check for colonization of SE in the cecum. Birds were given feed supplemented with TC (0.75%) or EG (1%) for 5 days before slaughter on d 42 for determination of SE populations in cecum and cloaca. The experiment was repeated twice. Trans-cinnamaldehyde and EG consistently reduced SE in the samples in both experiments ($P<0.05$). Body weights and feed consumption did not differ among the groups. The results suggest that TC and EG supplemented through feed could reduce SE colonization in market-age chickens and may provide a strategy for organic poultry producers. This program is funded by USDA-NIFA-OREI 2011-01955.

GOMP008

Expression analysis of genes potentially involved in host-microbe interaction of *Bifidobacterium bifidum* S17

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Tight adherence to intestinal epithelial cells (IECs) and the ability of successful colonization in the gastrointestinal tract (GIT) is discussed as a prerequisite for the beneficial effects of probiotic bacteria. Pili and fimbriae, proteinaceous structures of the bacterial cell surface, were shown to play an important role in the interaction of pathogenic bacteria with host tissues. More recently, pilus-like structures were identified in a range of bifidobacteria and were shown to be involved in colonization of mice by a *Bifidobacterium breve* strain.

We recently sequenced and annotated the genome of *B. bifidum* S17, a strain which shows high adhesion to cultured IECs and possess potent anti-inflammatory activity *in vitro* and in several murine models of colitis. Using bioinformatic analysis, four gene clusters potentially encoding for pili and further 9 genes for putative cell surface adhesins were identified. Expression of the genes was analyzed by semi quantitative reverse transcription (RT) PCR in bacteria grown *in vitro* in MRS in exponential or stationary growth phase.

Our results show that most of the genes are expressed, with higher expression rate in exponential growth phase. This correlates with increased adhesion of *B. bifidum* S17 harvested in the exponential growth phase to Caco-2 cells.

GOMP009

Individual *Salmonella* abundance in broiler caecum appears to reflect total microbiota composition and metabolism

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Introduction: In previous trials with experimentally *Salmonella* infected broilers we observed repeatedly that up to 20% of animals carry much less caecal *Salmonella* than the majority of birds. We hypothesized that initial variation of microbiota composition might contribute at least to some extend to the observed colonization inhibition.

Material & Methods: One group of 14 day old broiler chicks remained uninfected, while the second group was experimentally infected with a *Salmonella enterica* strain. Birds with the highest and lowest caecal *Salmonella* counts (7 d p.i.) were assigned to a so called "moderate" and "low" *Salmonella* group, respectively. Short chain fatty acid profile was determined, quantitative PCR was performed, %G+C profile analyzed and subsequently four characteristic %G+C fractions used for partial sequencing of 16S rRNA genes. In addition, comparable pools were subjected to an analysis by the CHICKChip, a chicken microbiota array.

Results: Challenged birds had significantly higher concentration of total SCFAs than the unchallenged birds. In the low *Salmonella* group propionic acid and in the moderate *Salmonella* group butyric acid was elevated. Quantitative PCR revealed that the total number of 16S rRNA gene copies was not significantly different between the groups. Total lactobacilli and clostridial cluster IV were significantly elevated in the challenged birds. In the unchallenged birds the major bacterial peak was at 46% G+C, whereas it was at a significantly higher and lower %G+C position in the low *Salmonella* and in the moderate *Salmonella* group, respectively. The majority of bacteria in the middle %G+C fractions belonged to the *Clostridium* cluster XIV. Nevertheless, the low *Salmonella* group showed a higher abundance of *Clostridium* cluster IV sequences and of three subgroups within cluster XIVa than the other two groups or birds. CHICKChip microarray profiling confirmed the enhanced relative abundance of cluster IV in the low *Salmonella* group.

Conclusions: We demonstrated a correlation between the microbial community structure and the *Salmonella* abundance. Future studies have to reveal whether the changed *Salmonella* numbers are due to changed microbiota composition and function or whether the extent of *Salmonella* colonization leads to these changes.

GOMP010

Molecular toolbox for functional analysis of bifidobacteria

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Bifidobacteria are inhabitants of the human gastrointestinal tract and some strains, such as *B. bifidum* S17, have been shown to exert a range of beneficial health properties including anti-inflammatory activity in colitis models (Preising *et al*., 2010). However, the exact mechanisms contributing to this still remain to be uncovered, because genetic modification of bifidobacteria remains a challenging field. That is why creation of the suitable molecular tools is necessary.

Hence we have set up a range of *E.coli-Bifidobacterium* shuttle vectors which differed in their antibiotic resistance, and plasmids pMGC and pMGE (with either chloramphenicol or erythromycin resistance) were selected as best for *in vivo* experiments, because they were stably maintained over 100 generations, did not affect *in vitro* growth rate of the cells, were cost effective for the large scale studies and allowed best selective recovery of *B. bifidum* S17 from the fecal samples. Indeed, survival of *B. bifidum* S17 containing pMGE or pMGC in the gut of C57BL/6J mice could be reproducible tracked.

We have further optimized a system for the stable and strong homologous and heterologous gene expression for bifidobacteria. A gus reporter gene assay was used to screen the range of promoters and pGAP promoter of *B. bifidum* S17 was shown to be the best suitable. Moreover, strong constitutive expression of the fluorescent proteins under control of pGAP *in vitro* and *in*

vivo, which allowed detection of recombinant bifidobacteria microscopically, strongly confirmed our results.
The obtained vectors allow expression of homologous and heterologous genes in bifidobacteria, so that in future the contribution of single genes and proteins to the particular probiotic effects of bifidobacteria could be studied in detail and ultimately generation of the recombinant probiotic strains with improved health promoting effects could be possible.

GOMP011

Effect of oxidized fat on the intestinal microbiota of rats

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The prevalence of obesity is increasing dramatically. One parameter that causes obesity is a high fat diet often with deep-fried products which is typical for the western style diet. Studies analysing the gut microbiota of obese and normal weight persons showed different microbial community structures in both groups. A high fat diet is thought to cause a higher prevalence for the development of obesity. Moreover deep fried products contain distinct lipid peroxidation products due to chemical reactions of the oil. The products are known to have a potent biological activity and thus may influence the microbial gut community.
To reduce the individual diet effect of persons we analysed the microbiota of rats fed with a standard diet supplemented with fat. Beside lean (fa/+) rats also obese diabetic (fa/fa) rats were analysed. 36 male obese diabetic rats (fa/fa) were divided into three groups: group 1 received a standard diet and fresh palm fat, group 2 the standard diet and oxidized palm fat, group 3 the standard diet and oxidized palm fat + a 10fold higher dose of vitamin E. The lean (fa/+) sugar rats received the standard diet and fresh palm fat. After two weeks feeding with the respective diet rat faeces were collected and the DNA was extracted out of it. The 16S rRNA gene was amplified using a universal bacterial primer modified for 454 pyrosequencing.

GOMP013

Intestinal microbiota composition of IL-10 deficient mice in relation to susceptibility to *Helicobacter hepaticus*-induced colitis

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The enterohepatic pathogen *Helicobacter hepaticus* (Hh) can induce inflammatory bowel disease (IBD) in mice with compromised immune response regulation. This system is often used as a model for chronic human IBD conditions such as ulcerative colitis (UC) and Crohn's disease (CD). However, IBD susceptibility can vary between different animal facilities.
IL-10 deficient C57/BL6J mice housed under specific pathogen-free (SPF) conditions at the animal facilities of Massachusetts Institute of Technology (MIT) develop robust typhlocolitis during Hh infection. In contrast, mice of the same strain kept at Hannover Medical School (MHH) do not develop any clinical or histological signs of colitis, despite being susceptible to Hh colonization. As this difference suggests that the composition of the intestinal microbiome might influence the induction of colitis during Hh infection, we investigated the composition of the intestinal microbiota of uninfected SPF IL-10-/- mice from both institutions using partial amplification of 16S rDNA genes with universal primers and subsequent deep sequencing with Roche/454 pyrosequencing technology.
Microbiota composition analysis was performed for caecum and colon samples from 8 uninfected mice from MIT and MHH, respectively, and for caecum samples from a second sample of 8 MHH mice. Microbiota composition differed markedly between MIT and MHH mice, and also between MHH mice reared in different years. Of the 119 non-rare operative taxonomic units (OTUs) identified in the dataset, 24 were only found in MIT samples, and another 13 OTUs could only be found in MHH samples. While most of the MHH-specific OTUs could only be identified to class or family level, the MIT-specific set contained OTUs identified to genus or species level.
We conclude that the difference of IL-10-/- mice from the two institutions in their susceptibility to Hh-induced pathology is associated with significant differences of microbiota composition. The data highlight the importance of characterizing the intestinal microbiome when interpreting the results from murine models of IBD.

IBV001

Re-evaluation of glycerol utilization within the species *Saccharomyces cerevisiae*

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Glycerol utilization has recently attracted renewed attention due to the large amounts of waste crude glycerol which are formed during biodiesel production. The current knowledge about the genetics and physiology of glycerol uptake and catabolism in *S. cerevisiae* is almost exclusively based on auxotrophic laboratory strains (which barely grow on glycerol) and in the presence of supplements that are either essential or facilitate glycerol growth. We tested the well-known lab strains S288c and CEN.PK (prototrophic versions) in synthetic medium (Verduyn medium) containing 6% (v/v) glycerol at pH 4.0. These lab strains used in many molecular studies of glycerol utilization could not grow at all under these conditions, but require complex medium composed of yeast extract and peptone or, at least, the addition of certain supplements for detectable glycerol growth. A screening of 89 *S. cerevisiae* strains revealed the existence of a few natural isolates that grow much better in glycerol than S288c and CEN.PK. The best isolate showed a maximum specific growth rate of about 0.1 h^{-1}. In order to check whether this strain uses the same pathway for glycerol utilization as characterized in S228c (via glycerol 3-phosphate as an intermediate) and not the alternative dihydroxyacetone pathway, we deleted the genes encoding for the major glycerol uptake protein (*STL1*), glycerol kinase (*GUT1*) and mitochondrial FAD^+-dependent glycerol 3-phosphate dehydrogenase (*GUT2*). All three deletion strains were unable to grow in Verduyn medium containing glycerol as the sole carbon source. This data confirms that Stl1p is the major transporter and the L-G3P pathway the sole pathway responsible for glycerol utilization even in the prototrophic strain of *S. cerevisiae* which shows significantly better glycerol growth. We are currently studying the genetic determinants allowing this improved glycerol growth in the best isolate.

IBV002

High-throughput microbial single cell analysis in picoliter bioreactors

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Recent improvements in bioreactor technology and analytical methods have provided increasing information about phenotypic heterogeneity in biotechnological processes. However, the interplay between environmental changes and cellular response is far from being completely understood. Lately we reported the successful utilization of a novel picoliter bioreactor (PLBR) for live-cell imaging of bacterial microcolonies [1]. Since then we have further improved the system by massive parallelization of picoliter sized reactor chambers.
In this contribution we present the development and application of a novel bioreactor system for high throughput data acquisition of bacterial growth and productivity at the single cell level. The presented system incorporates more than 1000 picoliter bioreactors allowing exceptional well environmental control as well as fast media exchange within seconds. The bioreactor height of approximately 1 μm restricts cell growth to a monolayer, ideal for microscopy. It is shown that the microfluidic system can be used to gain insights into industrial bioprocesses. The growth rate of *Corynebacterium glutamicum* WT was investigated in detail under various environmental conditions. Remarkably, *C. glutamicum* exhibited a higher growth rate compared to experiments performed at conventional lab scale. Furthermore, we investigated the L-valine productivity of single *C. glutamicum* ΔaceE cells utilizing the recently developed genetically encoded fluorescence based amino-acid reporter [2]. Interestingly, significant variability with respect to growth and productivity of single *C. glutamicum* ΔaceE cells was observed.

Our results prove that microfluidic single cell systems are powerful tools to further understand and eventually optimize bioprocesses.

[1] Grünberger *et al.*, A disposable picoliter bioreactor for cultivation and investigation of industrially relevant bacteria on single cell level. Lab on a Chip, 2012, 12, 2060-2068.
[2] Mustafi *et al.*, The development and application of a single-cell biosensor for the detection of l-methionine and branched-chain amino acids. Metabolic Engineering, 2012, 14(4), 449-457.

IBV003

Engineering a hyperthermophilic archaeon for temperature-dependent gene expression and bioproduct formation

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Hyperthermophilic microorganisms possess a great biotechnological potential; however, tools to genetically modify them have only been developed recently. We present here the first heterologous protein expression and the first induction system for protein and bioproduct production in the anaerobic archaeon *Pyrococcus furiosus,* which grows optimally at 100°C. We have engineered *P. furiosus* to switch its end products of fermentation in a temperature-controlled fashion without the need for chemical inducers. A recombinant strain (LAC) was constructed that expresses a gene (*ldh*) encoding lactate dehydrogenase from the plant biomass-degrading thermophile, *Caldicellulosiruptor bescii* (temperature optimum 78°C). Expression of the *ldh* gene was controlled by a "cold-shock" promoter that is up-regulated when *P. furiosus* cells are transferred from 98°C to 72°C. At 98°C the LAC strain fermented sugar to produce acetate, carbon dioxide and hydrogen as end-products and lactate was not detected. When the LAC strain was grown at 72°C or cells were rapidly cooled down to 72°C, the bacterial lactate dehydrogenase was expressed, resulting in up to 3 mM lactate being produced (lactate to acetate ratio 0.6-1.2). Subsequently, a new strain, LAC-2, was developed with a constitutive high transcription of *ldh*, increasing the lactate production by a factor of 3, and lactate now being the major end product. Generally, expression of genes from moderately thermophilic microbes in a hyperthermophile at temperatures at which the hyperthermophile has low metabolic activity provides a new perspective to engineering microorganisms for biomass degradation and biofuel formation.

Basen M, Sun JS, Adams MWW (2012). **Engineering a hyperthermophilic archaeon for temperature dependent product formation.** *mBio* 00053-12
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IBV004

Iron-dependent Regulation of Rhamnolipid Synthesis in *Pseudomonas aeruginosa* PAO1

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Pseudomonas aeruginosa is a gram-negative, opportunistic human pathogen that produces the biosurfactant rhamnolipid as secondary metabolites during stationary growth phase. The production of rhamnolipids by *P. aeruginosa* has become a prime example for the biotechnological production of biosurfactants and can serve as alternatives to the widely-used synthetic surfactants. However, a lack of understanding regarding metabolic and regulatory events and the absence of process control strategies with focus on optimized rhamnolipid production, the major drawback is the relatively low product yield. The aim of this project is to find strategies for the optimisation of rhamnolipid production with regard to large-scale industrial processes. Full comprehension of the molecular regulatory mechanisms behind rhamnolipid synthesis is key to manipulating and improving the rhamnolipid production capacities for commercial production.
The regulation of rhamnolipid synthesis is tightly governed by a complex regulatory network including bacterial quorum sensing (QS) systems as well as different sigma factors. Another interesting trigger for rhamnolipid synthesis besides cell density, nutrient availability and stress is iron starvation. The genes for mono- and di-rhamnolipid synthesis, rhamnosyltransferases 1 and 2 (*rhlA* and *rhlC*) respectively are encoded in one mutual operon, which is under the direct control of the *rhl* QS system and stationary phase sigma factor RpoS. The *rhl* QS system in turn is controlled by the *las* QS system and the nitrogen limitation sigma factor RpoN.
In the current study we present gene expression data of the systems that regulate rhamnolipid synthesis in *Pseudomonas aeruginosa* PAO1 during small-scale batch cultivation under different concentrations of ferrous iron using SYBR Green mediated quantitative real-time PCR. Iron starvation positively influenced the QS, i.e. the *las* regulon, as well as *rhlA* and *rhlC* expression, whereas iron shock caused a down-regulation of the rhamnolipid regulation circuitry. As ferrous iron showed a strong influence on rhamnolipid production, the effect of specific iron chelators, namely picolonic acid, lactoferrin and acetohydroxamic acid on the expression of the genes for rhamnolipid synthesis and its inherent regulatory circuitry was investigated.

IBV005

Metabolitesensors for rapid screening and single-cell isolation of small-molecule producing bacteria

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A major limitation in microbial strain-development for the industrial production of small-molecules is the subsequent screening for cells with increased production properties. Most small-molecules of interest are inconspicuous, and therefore have to be screened in laborious cultivations.
We here present a novel system to visualize the cytosolic concentration of small-molecules at the single cell-level of *E. coli* or *C. glutamicum*. The system is based on transcriptional regulators sensing intracellular concentrations of the molecule of interest, and in response drive transcription of an autofluorescent protein. We developed individual metabolitesensors to monitor the cytosolic concentrations of L-lysine, L-histidine, L-arginine, O-acetyl-serine, L-serine and L-leucine in *C. glutamicum* or *E. coli*. FACS together with high-throughput cultivation enables a number of novel applications, for instance the high-throughput isolation of an individual producer amongst billions of non-producers, or the screening of large plasmid libraries coding for modified variants of biosynthesis enzymes.
In one example we identified new chromosomal mutations leading to L-lysine overproduction. The wild type of *C. glutamicum* carrying the L-lysine sensor was treated with mutagen. Out of $6.5x10^6$ cells 200 L-lysine accumulating clones were selected using FACS within 30 minutes. Targeted sequencing identified 13 new chromosomal mutations in the known targets *lysC* and *hom*, whole genome sequencing unrevealed a *murE* mutation, which improved L-lysine titers significantly even in existing L-lysine producers.
In another example, error prone libraries of plasmid coded key enzymes for L-lysine- or L-arginine biosynthesis in *C. glutamicum* were screened in cells carrying the L-lysine or L-arginine sensor. Out of 10^6 cells, 100 clones were selected for each library using FACS within 5 minutes. Based on mutations identified in these mutants, saturation libraries and subsequent combinatorial libraries were analyzed in the same way, resulting in the isolation of highly feedback resistant mutants of those enzymes.
Since transcriptional regulators exist for sugars, sugarphosphates, vitamins, oxoacids, mevalonate, antibiotics, and further small-molecules, our technology is expected to boost metabolic engineering substantially.

Literature
Binder S. *et al., Genome Biol.* 2012, **13**(5):R40

IBV006

Carotenoid production in *Coryne-bacterium glutamicum*

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Carotenoids are yellow to red colored pigments originating from the terpenoid biosynthetic pathway. Carotenoids have been used in the feed, food and nutraceutical industries due to their antioxidative properties. Large-scale chemical synthesis is often difficult or costly and the isolation from the natural sources usually does not yield the desired quantities. For that reason the microbial biosynthesis is a promising approach for the production of terpenoids. *Corynebacterium glutamicum* contains the glycosylated C50 carotenoid decaprenoxanthin as yellow pigment. Starting from isopentenyl pyrophosphate (IPP), which is generated in the non-mevalonate pathway, decaprenoxanthin is synthesized via the intermediates farnesyl pyrophosphate, geranylgeranyl pyrophosphate, lycopene and flavuxanthin [1].
In this work, the bacterium *C. glutamicum* is analyzed with respect to the production of carotenoids. The yellow pigmented *C. glutamicum* possesses a carotenogenic gene cluster for the complete pathway of decaprenoxanthin synthesis starting from the precursors IPP and DMPP. We show that the genes of the carotenoid gene cluster *crtE*-cg0722-*crtBIYeYfEb* are co-

transcribed and characterize defined gene deletion mutants. However, the genome of *C. glutamicum* also encodes a second carotenoid gene cluster comprising *crtB2I2-1I2-2* shown to be co-transcribed, as well[2]. Experiments revealed that *C. glutamicum* possesses two functional phytoene synthases, namely CrtB and CrtB2. Furthermore we investigated *C. glutamicum* as a potential host for carotenoid production. Besides a considerable lycopene production by simple means of metabolic engineering also the non-native C50 carotenoid sarcinaxanthin could be accumulated in the cells.

[1] Krubasik *et al.* 2001
[2] Heider *et al.* 2012

IBV007
Synthetic microbial pathway for (*R*)-benzylsuccinate production

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Some denitrifiying, Fe(III) or sulfate reducing bacteria degrade toluene under anaerobic conditions. The first intermediate of the pathway is (*R*)-benzylsuccinate, an aromatic compound of potential biotechnological interest, e.g. in the production of polymers. We attempt to redesign the metabolism of standard bacteria such as *Escherichia coli* to establish the production of this intermediate in a synthetic process.
Synthesis of benzylsuccinate may either be started from the fermentation intermediate fumarate and exogenous toluene or from the fermentation product succinate and exogenous benzoate. We started our efforts with the production of benzylsuccinate from benzoate, using the toluene degradation pathway in reverse, since these steps can be catalyzed under aerobic conditions and all enzymes involved have been shown to be reversible.
To enter this reverse pathway, the precursor benzoate must be transported into the cytosol via a membrane-bound permease and activated to benzoyl-CoA. This has been established by introducing the genes for a benzoate transporter and a benzoate-CoA ligase from *Aromatoleum aromaticum*. This corresponds to a metabolic module for benzoyl-CoA generation, which is useful for many other biosynthetic purposes.
The reverse β-Oxidation cycle for benzylsuccinate production from benzoyl-CoA and succinyl-CoA was introduced by the cloned bbs-operon for beta-oxidation of benzylsuccinate from *Geobacter metallireducens*. First results on establishing the production of benzylsuccinate will be shown.

Boll M, Fuchs G, Heider J. (2002). Anaerobic oxidation of aromatic compounds and hydrocarbons. Curr Opin Chem Biol. 6(5):604-11.

IBV008
Development of an online reporter system for plasmid stability in *Saccharomyces cerevisiae*

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The biotechnological applications for yeasts like *S. cerevisiae* are constantly increasing and often plasmids play an important role, when heterologous gene expression is involved. However, the limited stability, i.e. loss of such plasmids is a serious problem. Therefore we want to develop a sensitive online reporter system to monitor plasmid stability in *S. cerevisiae.*
In this system expression of a chromosomally integrated reporter gene, whose gene product can readily be traced online is controlled by a plasmid encoded Cl lambda phage repressor. Thus plasmid loss will activate the reporter gene. Besides reporter signal strength tight regulation/repression of the reporter gene is desirable. This requests switch - like abilities in our knowledge not yet established for yeast promoters in that way.
Experiments addressing expression levels proposed the promoter of the yeast *PGK*1 gene (p*PGK*1) to be a suitable basis for assembly of a heterologous (semisynthetic) promoter controlling the reporter gene. By introducing multiples of modified lambda operator sequences in various positions tighter repression could be achieved. First experiments show that modifications so far analyzed do not affect p*PGK1* strength.
Effects of integration distant operator sites - probably allowing DNA loop forming in the yeast genome - on reporter gene expression were also studied and corresponding data will be presented. Furthermore the CI repressor has been modified in order to (a) locally increase its concentration by directing it to the host cell nucleus through addition of a nuclear localization signal; (b) prevent potential degradation through modification of the well-known peptidase recognition site implicated in phage activation. Further experiments have to be carried out, but this system shows promise to be used for synthetic gene networks enabling network logic approaches, too.

IBP001
Promoter Activity-Studies using a novel *in vivo* Reporter System for *Clostridium acetobutylicum* ATCC 824

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Fluorescent proteins such as the green fluorescence protein and its derivatives strictly require oxygen similar to luciferase-based reporter systems, which excludes these gentle *in vivo* reporters for applications in anaerobes. Recently, novel flavin mononucleotide (FMN)-based fluorescent proteins harboring light-oxygen-voltage domains were engineered for non-invasive reporter systems applicable for both aerobic and anaerobic conditions in *Escherichia coli* and *Rhodobacter capsulatus* (Drepper *et al.*, Nat. Biotechnol. 25:443-445). We have optimized these fluorescence-based reporters for monitoring gene expression in *Clostridium acetobutylicum*. To examine the applicability of the *in vivo* reporter system, we comparatively analyzed the promoter activities of four native *C. acetobutylicum* genes (*thlA*, *ptb*, *adc*, *hydA*). Furthermore, we studied the *adhE2* promoter activity in the wildtype and recently described knock-out mutants with an ethanologenic phenotype. The results demonstrated the effectiveness of this fluorescent reporter system to differentiate promoter strength and growth-dependent profiles.

IBP002
Agrobacterium tumefaciens-mediated transformation for *Aspergillus sojae*

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Aspergillus sojae is a well-known filamentous fungi, which has been mostly used in the production of the Japanese food "koji", by means of fermentation processes (Biesebeke et al. 2002). Protein production is also a relevant field with great potential for this organism. For example, the production of industrial important enzymes like pectinases by *A. sojae* has been studied in our group and by other groups (Demir, H., N. Gogus, et al. 2012). *A. sojae* is then certainly a promising biotechnological fungal strain, which is subject to study and optimization. In this sense, the development of a reliable transformation method for this fungus will help to exploit more widely the industrial potential of this organism. Furthermore such technique will greatly facilitate future molecular genetic studies of this fungus and allow us to gain a better understanding of the genetics of this organism.
In this study, we aim to setup a reliable transformation procedure for *A. sojae* strain ATCC 20235 based in the *Agrobacterium tumefaciens*-mediated transformation (ATMT) for filamentous fungi method (de Groot et al. 1998). This approach has been successfully applied for the introduction of DNA in diverse filamentous fungi (summarized in Wei and Chen, 2008 and Stark et al. 2011). ATMT is based in the capacity of *A. tumefaciens* to transfer part of its DNA (transferred DNA; T-DNA), contained in the tumor-inducing (Ti) plasmid, to the host cell. Such T-DNA is typically randomly inserted in the host genome as a single copy (Covert et al. 2001; Morioka et al. 2006). Up to now, we have designed the Ti vector “pRMegfp”, which incorporates a dominant antibiotic marker for *A. sojae*. This vector additionally incorporates the enhanced green fluorescent protein gene (*egfp*) in its T-DNA, a reporter gene that can be used as a second marker for gene expression. The completion of the pRMegfp vector construction and optimization of parameters relevant to transformation technique are central objectives to setup the first ATMT procedure for *A. sojae*, which as far as we know is lack for this fungus.

IBP003
Linalool dehydratase-isomerase of *Castella-niella defragrans* 65Phen: problems encountered during over-expression in *E. coli* and purification

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Monoterpenes, natural occurring hydrocarbons, are primarily produced and emitted by plants. These compounds are of climate relevance and well-known as essential oils and fragrances. In the atmosphere, monoterpenes are photochemically oxidized whereas microorganisms metabolize them under aerobic and anaerobic conditions. In order to be metabolized, pure hydrocarbons need to be activated. In the presence of molecular oxygen

these activations are mainly catalyzed by oxygenases. In the absence of oxygen alternative enzymes are needed.
C. defragrans 65 Phen, grown under denitrifying conditions, activates myrcene via stereospecific addition of a hydroxyl group. The so formed tertiary alcohol (S)-(+)-linalool is isomerized to the primary alcohol geraniol. Both reactions are catalyzed by the bifunctional enzyme linalool dehydratase-isomerase (LDI).
A purification based on metal-affinity chromatography was developed in order to study the enzyme in more detail. The *ldi* gene was N-terminally fused to an His_6-SUMO (small ubiquitin-like modifier)-tag. The histidine residues served as an affinity-tag for purification, while the SUMO-linker served as a protease cleavage site for tag-removal after purification.
We encountered several problems applying a general protocol. Elution of the fusion protein was hindered by tight binding to the column. Due to its high hydrophobicity the enzyme was vulnerable to aggregate during reduction of high imidazole and at higher protein concentrations. The poster will present solutions for those problems and an adapted protocol for the purification of the His_6-SUMO-LDI fusion protein.

IBP004
Identification of novel styrene oxide isomerases from soil bacteria for biotechnological aldehyde production

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Microorganisms from various phyla are able to metabolize styrene as a sole source of energy and carbon. A possible degradation pathway designated as '*side-chain oxygenation route'* was identified in several pseudomonads and rhodococci. As the initial step of the mentioned pathway, styrene monooxygenase oxidizes styrene to (*S*)-styrene oxide which is afterwards converted into phenylacetaldehyde by a styrene oxide isomerase (SOI). The aldehyde is further oxidized into phenylacetic acid by dehydrogenase activity and the formed acid can be degraded to intermediates of the tricarboxylic acid cycle. SOI genes (*styC*) can be found in most published styrene-catabolic gene clusters *styABCD* from pseudomonads and from *Rhodococcus* sp. ST-5 [1, 2].
Here we report on the isolation, identification, and biochemical characterization of novel SOIs from the genera *Rhodococcus*, *Sphingopyxis*, and *Sphingobium*. Especially in case of *Sphingopyxis* and *Sphingobium*, this is the first time that styrene degradation via side-chain oxygenation was shown and belonging SOIs were described. Growth rates of the isolated strains and the induction of SOIs were investigated. Furthermore, the substrate spectra and enzyme stability of enriched SOIs were determined. All enzymes were shown to be membrane-integrated and a procedure was developed to enrich the proteins in an active form to specific activities of up to 370 U mg^{-1} [1]. In combination with the independence of cofactors, the wide pH- and temperature tolerance, and the ability to convert other substituted styrene oxides among the native substrate, a biotechnological application of these enzymes could be imaginable [1].
Besides the biochemistry of novel SOIs also molecular genetic screening approaches for corresponding genes were successfully performed. That allows for the first time to describe the phylogenetic relation of SOI genes origination from different genera.

[1] **Oelschlägel, M.; Gröning, J. A. D.; Tischler, D.; Kaschabek, S. R. and Schlömann, M.(2012)** Styrene oxide isomerase of *Rhodococcus opacus* 1CP, a highly stable and considerably active enzyme. *Applied and Environmental Microbiology* **78:** 4330-4337.
[2] **Toda, H. and Itoh, N. (2012)** Isolation and characterization of styrene metabolism genes from styrene-assimilating soil bacteria *Rhodococcus* sp. ST-5 and ST-10. *Journal of Bioscience and Bioengineering* **113:** 12-19.

IBP005
The Physiologic Role of Pyruvate-Formate-Lyase in *Clostridium acetobutylicum*

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The Gram-positive *Clostridium acetobutylicum* has become a model organism for the acetone-butanol-ethanol (ABE) fermentation among solventogenic Clostridia.
To gain more insight in the biphasic fermentative metabolism, we used the ClosTron technology for targeted gene inactivation and generated knock-out mutants of the pyruvate-formate-lyase (*pflB*) and its activating enzyme (*pflA*). Pfl is catalyzing the reversible, coenzyme-A dependent and nonoxidative cleavage of pyruvate to acetyl-coenzyme-A. It has been demonstrated in a number of anaerobic bacteria, including many species of the genus *Clostridium*. The *pflB*-encoding gene CAC0980 is expressed in *C. acetobutylicum* during growth, as shown in a previous DNA time series microarray study. It was also detected in the proteome reference map of *C. acetobutylicum* ATCC 824. Since *C. acetobutylicum* contains neither a formate-hydrogen lyase as Enterobacteria, nor a formate dehydrogenase, the physiological role of Pfl in *C. acetobutylicum* remained obscure, due to the fact that so far no accumulation of formic acid in the growth medium has been observed. Therefore, it has been proposed that the clostridial enzyme may have a biosynthetic role.
This is supported by our observation that both mutants, in contrast to the WT, are not able to grow in minimal medium. However, when adding formate to these cultures the knock-out in the pyruvate-formate-lyase (*pflB*) and its activating enzyme (*pflA*) could be complemented. Since, the same was achieved by adding the purine nucleosides Adenosine and Guanosine we found out that formate synthesized by the pyruvate-formate lyase is used as a one-carbon-unit donor for the syntheses of the purine nucleotides.

IBP006
The incorporation of lysine in the cyanobacterial granule polypeptide (CGP) is responsible for the characteristic solubility behavior in recombinant *Escherichia coli*

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Cyanophycin, a highly-branched polypeptide, which is contained in the cyanobacterial granule, consists of a polyaspartic acid backbone to which arginine residues are linked by isopeptide bonds at free carboxylate groups and therefore called multi-L-arginyl-poly aspartic acid and CGP (1). CGP is a good candidate as starting-material for the production of nitrogen-containing bulk chemicals, as source for a polyaspartic acid-like polymer with reduced arginine content or for the production of β-dipeptides consisting of aspartate-arginine or aspartate-lysine by hydrolysis of the polymer with cyanophycinases. Until now there are numerous publications about the insoluble form, isolated from different recombinant bacteria strains, which consists of aspartate, arginine and up to 10 mol % lysine (2). We came to the conclusion that the solubility of recombinant insoluble CGP at pH 7 correlates with the temperature and the lysine content. Therefore, a minor lysine content (0-5 mol %) is related with solubility at high temperatures (100-80 °C), and a lysine content of 10-15 mol% dissolves CGP at 40-30 °C. These results demonstrate that isolated CGP is a mixture of different CGP derived polymers with different properties. In the past results about a soluble form, isolated from recombinant *E. coli* (3), engineered and recombinant yeasts (4) and recombinant *P. putida* (5) were published. Recently, we demonstrated that independent from the cphAs` origin a soluble derived CGP seem to be very prominent in recombinant *E. coli* strains using adjusted parameters. Solubility of CGP *in vivo* is associated with an increased content of more than 15 mol% lysine and can increase to over 25 mol% using cphA from *Synechocystis* sp. PCC 6308 for heterologous expression in *E. coli*. A proportion of more than 25 % (wt/wt) of CDM of the soluble form was never reported before. The latter can be isolated by a new low-cost, time-saving, effective and common isolation procedure.

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IBP007
Microbial synthesis of biopolymers from renewable resources

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In the last few years biopolymers from renewable resources moved into the center of research interest. Especially polyhydroxybutyric acid (PHA) is used to replace petroleum-based plastics. Materials gained from PHAs stand out for high resistance, variable mechanical properties and biodegradability. PHA-waste can relatively easy be degraded by microorganisms and goes as a substrate into the carboncycle.

In frame of a joint project the Institute of Microbiology and Wine Research (IMW) and the Test and Research Institute Pirmasens e.V. (PFI) developed a method for the production of PHA from the renewable resource "wheat straw".
After a thermohydrolytic process and an enzymatic treatment of the substrate about 90% of the sugars in the straw became soluble. The obtained main sugars glucose and xylose were used as carbon source by the microorganisms, which were tested for PHA production. In this procedure both sugars were separated in two different fractions. Several microorganisms from the strain-collection of the IMW were found to produce PHAs from the substrates glucose and xylose.
Another approach was a two-step process for the production of PHA from the xylose-fraction of the wheat straw. This technique used an intermediate product as substrate for the synthesis of PHA.
Straw is a renewable resource and is produced in large quantities as a byproduct in the food production. Therefore, its efficient use for the synthesis of biopolymers could improve significantly the economic and ecological balance compared to petrochemical methods.

IBP008
MazProTec – Two-Step-Strategy towards an Orthogonal Bio-System

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The integration of orthogonality is a crucial research strategy in the rational design of biological systems for novel applications[1]. In order to produce such highly predictable orthogonal systems, we propose to employ cell-free systems of highly engineered composition generated from living cells, which are complex enough to reproduce the major synthetic capabilities of living cells - such as the synthesis of natural and artificial saccharides - but are simplified enough to come close to truly engineerable systems[2]. We propose a two-step-strategy: In a first step, the protein synthesis of a growing bacterial cell will be channeled solely to a limited set of system components with the help of the RNA-interferase MazF[3]. In a second step, the cells will be homogenized and the resulting cell-free extract, already enriched in the required protein components, will be subjected to selective hydrolysis of predefined proteins, which otherwise would connect the designed system to the remaining protein background, which would make the performance of the complex system unpredictable. As a proof-of-concept, we propose to implement a preparative 12-step synthesis from cheap glucose and N-acetyl-glucosamine to a valuable antiviral-precursor, N-acetyl-neuraminic acid.

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IBP009
Balanced overexpression of isopenicillin N acyltransferase leads to increased penicillin production in *P. chrysogenum* production strains

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Intense classical strain improvement has yielded industrial *Penicillium chrysogenum* strains that produce high titers of penicillin. These strains contain multiple copies of the penicillin biosynthesis cluster encoding the three key enzymes: δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine synthetase (ACVS), Isopenicillin N synthase (IPNS) and isopenicillin N acyltransferase (IAT). The phenylacetic acid CoA ligase (PCL) gene encoding the enzyme responsible for the activation of the side chain precursor phenylacetic acid is localized elsewhere in the genome in a single copy. Since the protein level of IAT already saturates at low cluster copy numbers, IAT might catalyze a limiting step in high yielding strains. Here, we show that penicillin production in high yielding strains can be further improved by the overexpression of IAT while at very high levels of IAT the precursor 6-aminopenicillic acid (6-APA) accumulates. Overproduction of PCL only marginally stimulates penicillin production. These data demonstrate that in high yielding strains IAT is the limiting factor and that this limitation can be alleviated by a balanced overproduction of this enzyme.

IBP010
Production of natural pigments from novel local psychrotolerant *Kocuria* sp

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Two psychrotolerant coloured bacteria, *Kocuria polaris* and *Kocuria carniphila*, which were isolated from Nile river during winter in Kafer El-Shakhe- Egypt. They were investigated for natural pigments production as *K. carniphila* was a yellow isolate and *K. polaris* was an orange one.
These natural pigments were extracted using acetone after cultivating both isolates on King's medium and being incubated at 15°C for 5 days. Low incubation temperatures for both bacterial isolates were found to enhance production of large amount of pigments which was seen to be reduced by increasing temperature till it was completely vanished above 30^{0}C. Light was found to be suppressive for pigmentation in both isolates where in dark places pigment production was highly improved rather than in lightened ones. On the other hand**, c**hange in pH values didn't affect pigmentation in both strains.
Bacterial pigments were then identified by TLC, where it was found that *K. carniphila* contains β-carotene and xanthophylls, while *K. polaris* contains β-carotene and echinenone.
Pigments were then separated in column chromatography and it was found that *K. carniphila* contains 0.0127 mg/g of β-carotene and 4.06 mg/g xanthophiles while *K. polaris* contains 0.0308 mg/g of β-carotene and 0.02 mg/g of echinenone. β-carotene, echinenone and xanthophylls were determined spectrophotometricaly after separation.

Key Words: psychrotolerant bacteria, *Kocuria carniphila, Kocuria Polaris,* natural pigments.

IBP011
Platform engineering: *Corynebacterium glutamicum* with attenuated pyruvate dehydrogenase complex-activity for the production of L-valine, 2-ketoisovalerate and L-lysine

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Corynebacterium glutamicum is a facultative anaerobic, Gram-positive organism that grows on a variety of sugars and organic acids and is the workhorse for the production of a number of amino acids, such as L-glutamate, L-lysine and L-valine. Replacement of the native promoter of the *aceE* gene encoding the E1p enzyme of the pyruvate dehydrogenase complex (PDHC) by mutated *dapA* promoters (1) led to a series of *C. glutamicum* strains with gradually attenuated PDHC activity. Additional overexpression of the *ilvBNCE* genes encoding the L-valine biosynthetic enzymes acetohydroxy acid synthase, isomeroreductase, and transaminase B (TA), resulted in L-valine overproduction in all strains. *C. glutamicum aceE* A16 (pJC4ilvBNCE) showed the highest L-valine yield and was further improved by additional deletion of the *pqo* and *ppc* genes encoding pyruvate:quinone oxidoreductase and phosphoenolpyruvate carboxylase, respectively. In fed-batch fermentations *C. glutamicum aceE* A16 Δ*pqo* Δ*ppc* (pJC4ilvBNCE) produced up to 86.5 g l^{-1} L-valine with a volumetric productivity of 1.6 g l^{-1} h^{-1} and a yield of about 0.24 g per g of glucose. The additional deletion of the *ilvE* gene encoding TA transformed the L-valine producer into an efficient 2-ketoisovalerate producer, secreting up to 35 g l^{-1} 2-ketoisovalerate from glucose in fed-batch fermentations. Moreover, the attenuation of the PDHC in the L-Lysine producer *C. glutamicum* DM1933 also improved L-lysine production. In summary, *C. glutamicum* strains with attenuated PDHC show no auxotrophy for acetate and a drastically improved production performance compared to producer strains with inactivated PDHC and therefore, represent an excellent platform for metabolic engineering approaches.

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IBP012

Identification of essential amino acid residues of the Acetolactate Synthase from *Bacillus subtilis*

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Isobutanol is a hydrophobic alcohol, with significant importance as renewable future biofuel and chemical building block. Several groups are involved in the quest of producing hydrophobic alcohols, such as isobutanol. The race is on to produce this chemical building block by efficient biotechnology processes, where genetically engineered organisms play a central role.
However, in artificial isobutanol biosynthesis pathways, a key enzyme is the thiamin diphosphate dependent acetolactate synthase (Als).
Als catalyzes the decarboxylation of one molecule pyruvate and transfers the remaining cofactor-bound hydroxyethyl group to a second molecule of pyruvate producing 2-acetolactate.
It was recently found that 2-ketoisovalerate is an alternative substrate of *Bacillus subtilis* Als (AlsS), which together with pyruvate acts as a key intermediate in artificial isobutanol biosynthesis pathways.
Catalyzed reaction 1: 2 Pyruvate --> 2-Acetolactate + CO_2
Catalyzed reaction 2: 2-Ketoisovalerate --> Isobutyraldehyde + CO_2
Here we report the crystal structure of *B. subtilis* acetolactate synthase for the first time. The x-ray crystal structure revealed AlsS to be a homotetramer which predominantly consists of alpha helical structures, comprising four large subunits, each having a molecular weight of 63 kDa.
The structure incorporates two cofactors, TPP and $MgCl_2$. Based on crystal structure information we identified possible amino acid residues essential for the catalytic activity of AlsS. Resulting mutants were characterized with respect to pyruvate and ketoisovalerate substrate selectivity and thermal stability.
Future work involves identification of other enzyme variants with higher activity towards ketoisovalerate compared to wild type AlsS.

IBP013

Natural products from marine fungi for the treatment of cancer

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Marine fungi are a highly potent group of secondary metabolite producers, though they are not well studied and in particular underutilised in terms of biotechnological application. Here, we demonstrate the sustainable exploitation of marine natural resources providing appropriate culture conditions for the group of marine fungi, thus enabling efficient production of marine natural products in the laboratory and also in large scale cultures, avoiding harm to the natural environment. In the focus are new anti-cancer compounds. These compounds will be characterised to the stage of *in vivo* proof of concept ready to enter further drug development in order to valorise the results of the project.
Two approaches are used to gain effective producer strains:
a) Candidate strains originating from our unique strain collection of marine fungi are characterised and optimised using molecular methods.
b) New fungi are isolated from unique habitats, i.e. tropical coral reefs, endemic macroalgae and sponges from the Mediterranean. Culture conditions for these new isolates are optimised for the production of new anti-cancer metabolites.
We develop a process concept for these compounds providing the technological basis for a sustainable use of marine microbial products as result of "Blue Biotech". Therefore, we will explore the potential of marine fungi as excellent sources for useful new natural compounds along the added-value chain from the marine habitat to the drug candidate and process concept.

IBP014

Towards the biobased production of C5 and C6 dicarboxylic acids in a recombinant *Escherichia coli* strain

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Glutaric acid (pentanedioic acid) might be used for polyester synthesis, related to the biodegradable Ecoflex (BASF). We produced glutaconic acid (pent-2-enedioic acid) in a recombinant *E. coli* strain expressing six genes from the 2-hydroxyglutarate pathway of glutamate fermentation in *Clostridiales* (1). For the reduction of glutaconyl-CoA to glutaryl-CoA we expressed the genes encoding the non-decarboxylating glutaryl-CoA dehydrogenase (Gdh) and the electron-transferring flavoprotein (EtfAB) from *S. aciditrophicus* in *E. coli*. We suppose that Gdh together with Etf catalyzes the electron bifurcation of 2 NADH to glutaconyl-CoA and ferredoxin yielding glutaryl-CoA and reduced ferredoxin.
To produce dicarboxylic acids with six carbons, we will use the same enzymes starting from 2-oxoadipate via (*R*)-2-hydroxyadipate and its CoA-thioester that is dehydrated to homoglutaconyl-CoA followed by reduction to adipoyl-CoA (2). Adipate together with 1,6-diaminohexane is polymerized to Nylon-6,6®. To convert 2-oxoglutarate to 2-oxoadipate three enzymes from yeast involved in lysine biosynthesis have to be additionally introduced into *E. coli*. To avoid the simultaneous production of glutarate in this recombinant *E. coli* strain, the (*R*)-2-hydroxyglutarate dehydrogenase has to be exchanged by a specific (*R*)-2-hydroxyadipate dehydrogenase (2,3).

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IBP015

Characterization of all membrane-bound dehydrogenases from *Gluconobacter oxydans*

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G. oxydans, as all acetic acid bacteria, has several membrane-bound dehydrogenases with often broad substrate spectra which oxidize a multitude of alcohols and polyols in a stereo- and regio-selective manner. Many different oxidative activities have been purified from various acetic acid bacteria in the past, but in most cases without reporting associated sequence information. We constructed a set of strains with consecutive clean deletions of all membrane-bound dehydrogenases in *G. oxydans* 621H using a new method for marker-less clean deletions in *G. oxydans*. Then we studied the substrate oxidation spectrum of these strains by using a newly developed whole cell DCPIP activity assay in microtiter plates. This allowed a detailed and comprehensive characterization of the substrates oxidized by each membrane-bound dehydrogenase. The assays revealed that general rules can be established for some of them and extended the known substrate spectra of several enzymes. It was also possible to assign former reports of purified proteins to their corresponding genes. We showed that there are less membrane-bound dehydrogenases in *G. oxydans* than expected and that the deletion of all of them is not lethal for the organism. The results were completed by expression of the individual dehydrogenases in a newly established protein expression system for *G. oxydans* in the multi-deletion strain and determination the oxidation spectrum. This expression system was also used to express and characterize metagenomic membrane-bound dehydrogenases from a mother of vinegar.

IBP016

The Role of Cysteine for Acetogenesis and Solventogenesis of *Clostridium acetobutylicum*

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The batch fermentation of *Clostridium acetobutylicum* is characterized by a acetogenic growth phase during exponential growth when mainly acetate and butyrate are fermentation products. Then, at the end of exponential growth and during stationary phase phase, the organism switches to solventogenic growth and large amounts of acetone, ethanol and butanol are produced. These growth phases can be studied independent from each other in a phosphate-limited continuous culture. In transcription analysis of continuous cultures using DNA microarrays it became evident that, among others, operons involved in sulfur assimilation are strongly up-regulated during solventogenesis. Using the ClosTron technique we constructed two knock-out mutants in the genes CAC0105 and CAC0930 annotated as involved in sulfate reduction and cysteine biosynthesis. We did complementation experiments with sulfite and cysteine to prove the predicted function and show that more reduced sulfur is needed by the organism during solventogenesis. This could be due to synthesis of a high concentration of a sulfur containing redox mediator like e.g. thioredoxin needed during solventogenesis. Construction and analysis of further

mutations in sulfur metabolism try to narrow down the sulfur compound involved and its physiologic function.

IBP017

Magnetosome display of heterologous fusion proteins in *Magnetospirillum gryphiswaldense*

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Magnetosomes are membrane-enclosed magnetic organelles produced by magnetotactic bacteria. The biomineralization of these unique nanostructures, which consist of magnetite (Fe_3O_4) crystals, is under strict genetic control. Thus, genetic engineering can be employed for the biosynthesis of magnetic nanoparticles with tuned characteristics. Due to encapsulation of the magnetite core within the magnetosome membrane, also the magnetosome surface is accessible to functionalization by genetic engineering, which makes magnetosomes an interesting and promising tool in fields like (bio)nanotechnology, synthetic biology, or even theranostic nanomedicine.
MamC is the most abundant and tightly bound protein in the magnetosome membrane in *Magnetospirillum gryphiswaldense*, which makes it the perfect anchor for magnetosome display of hybrid proteins by translational fusions.
To ensure high yields of functional magnetosome fusions and to reduce potential toxic effects of foreign proteins, several inducible expression systems for were tested for the production of functionalized magnetosomes. To this end, different operator and repressor sequences were analyzed and optimized using a codon optimized version of GFP as reporter for heterologous expression. This resulted in a high and stable magnetosome expression of GFP fusions, which are useful for tracking intracellular magnetosome protein localization, as well as bimodal (fluorescent and magnetic) markers for MRI applications.
Besides GFP, fusions with other proteins and peptides are being explored, such as nanobodies and biomineralizing peptides. Magnetosomal expression of functional nanobodies resulted in intracellular recruitment and repositioning of proteins tagged with their cognate epitopes. This results in phenotypic effects on motility and magnetosome chain assembly.
In addition, the magnetosome functionalization with several biomineralizing peptides is currently tested, which may lead to the production of new magnetic hybrid materials, which might have an application as drug delivery agents or as sensors.

IBP018

Relative abundance of *Nitrotoga* in a biofilter of a freshwater aquaculture plant

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Recirculation aquaculture systems (RAS) gain increasing attention for the production of fish and other aquatic organisms. The re-use of water is of ecological importance, but requires biological filters to remove toxic ammonia and nitrite. Nitrification is promoted on the surface of carrier elements, in this case located in well-aerated moving bed reactors. The carrier elements (plastic material) are colonized by ammonia and nitrite oxidizing bacteria (NOB), grown in a dense biofilm together with heterotrophic bacteria. In most aquaculture systems (marine and freshwater), *Nitrosomonas* and *Nitrospira* were identified as main ammonia and nitrite oxidizers.
In this study, a RAS used for the production of rainbow trouts was driven at a temperature of 13°C and pH 6.8. Community analyses of nitrifying bacteria revealed a coexistence of *Nitrospira* and the recently discovered *Nitrotoga* for the second step of nitrification. *Nitrotoga* is a cold-adapted bacterium, which was initially found in permafrost-affected soils in Siberia [1]. Later on, it was also shown to be engaged in the purification of wastewaters in European WWTPs [2]. The here detected representative revealed a 99.9% similarity of the 16S rRNA gene to *Nitrotoga* sp. HAM-1 from activated sludge. This nitrite oxidizer occurred together with *Nitrospira*, being 99.1% respectively 99.7% similar to *Nitrospira defluvii*. In the biofilm samples, both NOB clustered in dense microcolonies in close vicinity to the ammonia oxidizer *Nitrosomonas*.
The conditioning of a new moving bed biofilter resulted in a reduced abundance of *Nitrotoga* in favour of *Nitrospira* and later on *Nitrobacter*. The change in the nitrite oxidizing community was documented by specific PCR as well as cloning of the 16S rRNA gene, electron microscopy and FISH.
Nitrite oxidizing enrichment cultures from the freshwater aquaculture plant were physiologically investigated to elucidate the influence of single operational parameters like temperature and pH. Reference cultures of *Nitrospira* and *Nitrotoga* were used to confirm separation of both NOB into distinct ecological niches. In addition to the low temperature of 13°C, the low pH value of 6.8 seems to be of advantage for a selective enrichment of *Nitrotoga*.

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IBP019

Improved recombinant protein production in *Bacillus megaterium* by coproduction of proteins identified via transcriptome analyses

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Since many years the Gram-positive microorganism *Bacillus megaterium* is used for the production and secretion of recombinant proteins. Although optimized promoter systems as the xylose-inducible one, improved strains and adapted cultivation conditions are available resulting in intracellular protein amounts of more than one mg per liter [1, 2], there are still bottlenecks visible, especially in the secretion process of recombinant proteins.
Here, some of these bottle necks were identified via transcriptome analyses in form of strongly up- or down-regulated expression of involved genes when comparing recombinant and wild type strains. The expression of certain genes was found to be more than 200 fold up- (PrsA-like protein) or 70 fold down-regulated (ribosomal protein). Genes, whose expression was strongly regulated, were used as targets for xylose-inducible vector-encoded coexpression together with target genes coding for the intracellular green fluorescent protein (GFP) and the antibody fragment D1.3 scFv [3], respectively. The coexpression of single target genes resulted in product increase of more than 3.2 fold which could be established in shake flasks cultivation and improved under high cell density conditions. When combining different target genes in artificial operon structures the secretion of recombinant antibody fragment could be enhanced even up to five fold. Finally, for the first time, the antibody fragment encoding gene could be cloned under control of the optimized xylose-inducible promoter. In combination with coexpression of the artificial operons combining the single target genes this could further increased the secretion of the antibody fragment.

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IBP020

Effects of the fungicide carboxin on growth, succinate dehydrogenase activity and succinate production of the non-conventional yeast *Yarrowia lipolytica*

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The apathogenic yeast *Yarrowia lipolytica* belongs to the well-studied 'non-conventional' yeast species. Its ability to secrete various organic acids, like pyruvic, citric, isocitric and alpha-ketoglutaric acids, in large amounts is of interest for biotechnological applications. *Yarrowia lipolytica* also secretes succinate in low amounts. The fungicide carboxin shows a specific effect on basidiomycetal fungi, but also on some representatives of the classes *Ascomycetes*, *Deuteromycetes* and *Phycomycetes*. This effect was explained by an inhibition of succinate dehydrogenase through binding of this

antibiotic to the ubiquinone binding site causing a prevention of ubiquinone reduction. Because succinate dehydrogenase is part of the respiratory chain (complex II) and citric acid cycle an inhibition of this enzyme might also lead to fatal effects on *Yarrowia lipolytica* cells. We have studied the effect of carboxin on growth, succinate dehydrogenase activity and succinate production in this yeast. We observed a reduction of growth and succinate dehydrogenase activity depending on the used carboxin concentration. However, the most remarkably result was an increase of succinate production after addition of a certain concentration of carboxin, probably due to the reduction of succinate dehydrogenase activity.

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IBP021

Genetic engineering of the Colombian strain *Clostridium* sp. IBUN 158B for improving the bioconversion of glycerol into 1,3-propanediol

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The non-pathogen strain *Clostridium* sp. IBUN 158B, isolated from a tomato field in Colombia and apparently belonging to a new species, is able to transform raw glycerol from a biodiesel production process into 1,3-propanediol (1,3-PD), a substrate for textile polymers. In this work, the strain *Clostridium* sp. IBUN 158B was modified by genetic engineering in order to reach an industrially efficient 1,3-PD production level. The genes encoding the enzymes hydrogenase (*hydA*), 3-hydroxybutyryl-CoA-dehydrogenase (*hbd*), and lactate dehydrogenase (*ldhA*) were inactivated in single inactivation mutants with the aid of the ClosTron mutagenesis system (Heap *et al*., 2009). In this way, more NADH generated during the oxidative metabolism should be available for 1,3-PD production via the glycerol reductive pathway. Additionally, the genes *dhaB1*, *dhaB2*, and *dhaT*, constituting the glycerol reductive pathway and clustered together in the related strain *Clostridium* sp. IBUN 13A, were ligated in the vector pMTL007C-E2 (Heap *et al*., 2010) under the control of the constitutive P_{fdx} promoter of *C. sporogenes*. The resulting overexpression plasmid was conjugated in *Clostridium* sp. IBUN 158B WT as well as in the inactivation mutants *hydA*-420s::Ll.LtrB and *ldhA*-508s::Ll.LtrB, with the aim of funneling more glycerol to 1,3-PD production. The performance of each resulting strain was evaluated in 80-ml batch cultures during 72-120 h using a complex medium with 217 mM glycerol as substrate. The plasmidless inactivation mutants *hydA*-420s::Ll.LtrB and *hbd*-414s::Ll.LtrB reached lower cell concentrations and 1,3-PD titers than *Clostridium* sp. IBUN 158B WT, while all strains containing the overexpression plasmid produced significantly more 1,3-PD than the WT strain.

The best 1,3-PD producers were the WT strain carrying the overexpression plasmid and the plasmidless *ldhA* mutant, both reaching significantly higher cell concentrations and approx. 3-fold higher 1,3-PD titers (56-58 mM) than the plasmidless WT strain. Fermentations in reactors under controlled conditions should be carried out with these strains in order to ascertain their maximal 1,3-PD production potential.

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IBP022

Does the host matter? Comparative expression study of lipase CalA in yeast systems

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Yeasts are versatile hosts for certain gene expressions, as they combine the ease of genetic manipulation with the capability to execute eukaryotic processing steps [1]. Comparative studies on protein expression have usually focused on the productivity of different yeast strains, whereas an impact of the host selection has rarely been published [2], although an influence of different posttranslational processing on the biochemical properties of the produced enzyme is conceivable.

Based on a previous comparative review [1], three yeasts species, *S. cerevisiae*, *K. lactis* and *H. polymorpha* (patented yeast strain, derived from ARTES Biotechnology GmbH), were chosen for this project. As a proof of principle, the lipase CalA from *C. antarctica* was selected for this study. This lipase was shown to have encouraging properties as a biocatalyst, e. g., the ability to accept highly branched substrates [3]. As reported already, CalA was functionally expressed in *E. coli*, but only with co-expression of chaperones [4], and high yields were obtained with the fungi *A. oryzae* [5] and *P. pastoris* [6].

Using a codon optimised gene sequence, CalA was expressed intracellularly in *S. cerevisiae* and in secreted form in *K. lactis* and *H. polymorpha*. Application of the enzyme produced in the three different hosts revealed interesting results in the qualitative and quantitative comparison as well as in further characterisation of this lipase, e. g., by screening with different substrates of chemical interest.

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IBP023

Direct micro-encapsulation of probiotic *Lactobacillus reuteri* from slurry-fermentation with whey protein

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For the encapsulation of probiotics by spray-drying different hydrocolloid or protein matrices are available to date. Dietary whey protein is a healthy but cheap waste product from cheese production which is metabolized by lactic acid bacteria, dispersible in water, hygroscopic, acid-resistant and thermostable. In order to increase process efficiency and to improve the viability of bacteria this study aimed at developing a micro-encapsulation of *Lactobacillus reuteri* with whey protein directly from slurry fermentation.

Log3 CFU/ gram *L. reuteri* were inoculated in watery 20 % (w/v) whey solution (pH 6.0) with or without 0.5 % (w/v) yeast extract as supplement and cultivated at 37 °C in a 200 mL batch slurry fermentation. The growth of bacteria was determined at 24, 48 and 72 h culturing time. Whey solutions containing at least log8 CFU/ gram bacteria were spray dried at 55 °C and 65 °C outlet temperature with a flow rate of 500 NL/ h, respectively. Release and viability of encapsulated bacteria were detected by inoculating the dried powder into artificial gastric (pH 1.9 with pepsin) for 30 min and thereafter into intestinal juice (pH 7.5, with pancreatin and bile salt) for at least 5 h.

The growth of *L. reuteri* increased in whey solution from log7 up to log8 CFU/ g after 48 h after yeast extract supplementation. In a stable spray-drying process particles sized 5.3 μm +/- 0.5, with 7 % +/- 1 moisture, and with a bacteria content of at least log6 CFU/g were produced. Both outlet temperatures neither influenced the particles size and moisture nor the bacteria content. Processed bacteria examined a loss of log1 CFU/ gram under simulated gastrointestinal conditions. Non-encapsulated ones reveal log4 CFU/ mL loss.

In conclusion, whey protein utilized as bacterial substrate and encapsulation matrix within a direct fermentation and spray drying process might offer an efficient production option for vital probiotics.

IBP024

Single cell growth under constant environmental conditions and defined C sources: a case study for *Corynebacterium glutamicum*

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Single cell analysis is a growing field for the investigation of metabolic processes. Recently we reported on a novel picoliter bioreactor (PLBR) to study growth and productivity of bacteria on single cell level [1, 2]. Cell growth and morphology can be tracked by automated time-laps microscopy.

For the first time detailed single cell growth investigations are shown to gain further insights in cell metabolism. In fact, we cultivated *Corynebacterium glutamicum* on well-defined media compositions using a variety of single C sources. Compared to typical cultivation systems growth can be investigated under constant environmental conditions without the effect of accumulating byproducts and changing medium compositions, as omnipresent in typical batch systems. This is due to the continuous flow operation mode of our PLBR device, in which secreted products and byproducts are instantly removed by diffusion and laminar flow. In detail, *Corynebacterium glutamicum* cells were provided with various single C sources, ranging from

sugars (e.g. fructose), organic acids (e.g. acetate) to metabolites of the central metabolism (e.g. pyruvate, citrate). Inside the PLBR there is a tendency that growth on different C sources is faster, than compared to conventional batch cultivations with the identical medium. It is assumed that the results of typical batch cultivations are masked by the changing environmental conditions such as byproducts or changing substrate concentrations. The maximum growth rate decreases from 0.6 1/h on e.g. glucose to growth rates of < 0.3 1/h on carbon metabolites of the TCA.
In order to understand the effects of co-utilization and the influence of byproducts onto the overall growth rate, currently combinations of different C sources with defined amounts of byproducts are used as growth media.

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IBP025
Fermentative production of biopolymers based on wheat straw

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With a global rising energy demand and a shortage of fossil energy sources, the importance of renewable raw materials has been increasing from year to year. Especially the importance of lignocelluloses from residual biomass is growing. Today, most of the renewable raw materials are used for the bioethanol production. Furthermore it will become necessary to replace also the petrochemical plastics through biopolymers based on agricultural by-products. Different biopolymers are known. Especially the group of polyhydroxyalkanoate (PHA) plays an import role, which based on their good product properties which are very similar to petrochemical polymers like polyethylene (PE). The well-known representative of the PHAs is the polyhydroxybutyric (PHB).In a joint project of the Test and Research Institute Pirmasens (PFI) and the Institute of Microbiology and Wine Research (IMW) a process will develop for the production of PHB from residual biomass like wheat straw. Based on a thermal pressure hydrolysis (TPH) and a subsequent enzymatic hydrolysis step, about 90 % of the contained sugars become soluble from the wheat straw. The amount of glucose from the hydrolyzed cellulose fraction reached up to 420 kg glucose per ton referring to dry mass wheat straw.Several microorganisms are known for their ability to produce PHB from glucose, i.e. *Cupriavidus necator*. Although the enzymatic hydrolysate contain some growth nutrients, an additional supplementary was necessary for a sufficient growth and large PHB yields. Thus one major goal was the establishment and optimization of the fermentation process by different PHB producing microorganisms based on glucose in wheat straw hydrolysates.

IBP026
The effect of pSOL1 and an uncharac-terized *etfAB* dependent dehydrogenase on the metabolism of *Clostridium acetobutylicum*

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The genome of *Clostridium acetobutylicum* consists of the chromosome as well as a 210-kb pSOL1 megaplasmid coding for genes of acid uptake and solvent formation. The exact influence of pSOL1 on expression of chromosomal genes is not yet known. Using the ClosTron method, we constructed a pSOL1-free strain and then characterized it in a phosphate-limited continuous fermentation. Results revealed that the pSOL1-free strain survives a shift to pH 4.5 in a phosphate limited chemostat culture well, after some adaptation, contradicting the hypothesis that pSOL1 is necessary to survive at this pH due to the acid uptake and solvent formation function encoded by the megaplasmid. *C. acetobutylicum* contains a second chromosomal coded *etfAB* dependent dehydrogenase (CAC2542) of unknown function. A knock-out mutant of this gene was characterized in continuous fermentations. Compared with the wild type strain the mutant culture produced a higher concentration of butyrate during acidogenic growth, a lower concentration of butanol during solventogenic growth at low pH and needs some time for adaptation during the shift. This phenotype might be explained by an inhibition of external butyrate uptake and is interesting due to a possible bioenergetic role of the *etfAB* dependent dehydrogenase. Its phenotype resembles a knock-out mutant in the yet uncharacterized regulator CAP0129 encoded on the megaplasmid, suggesting that this gene might also play a role in butyrate assimilation. Physiologic data and transcription analysis of the described strains are presented.

IBP027
Non-standard amino acids as synthons for pharma-ceuticals: novel substrates and procedures for a modified hydantoinase process

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The hydantoinase/carbamoylase system is well established for the industrial production of enantiopure D-configurated alpha-amino acids with unnatural residues, e.g. D-phenylglycine and *p*-hydroxy-D-phenylglycine as side chains of the semisynthetic penicilline derivatives ampicillin and amoxicillin.
The use of immobilized enzymes instead of whole-cell catalysts led to a reduction in byproducts and waste [1]. However, this process is limited to few products [2].
We are trying to modify the hydantoinase process in several ways:
- Application of dihydrouracils as substrates to also obtain beta-amino acids [3].
- Application of immobilized enzymes in a microfluidic system with *in situ* product recovery.
- The resulting possibility to use different solvents in one system will broaden the substrate (and solvent) variety.

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IBP028
Investigation of Xylose Metabolism in *Clostridium acetobutylicum* ATCC824 and *Clostridium saccharo-butylicum* NCP262

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D-Xylose is a very common pentose sugar present in plant cell walls. It is an important substrate component for production of biofuels and solvents by solventogenic *Clostridia* from lignocellulose. The detailed understanding of the xylose metabolism is a prerequisite for the optimization of those fermentation processes. We have shown in a former study that there are two xylose-induced operons in *C. acetobutylicum*: The first one is being repressed by the presence of glucose. It was not expressed until nearly all glucose was consumed as in catabolite repressed systems. The second operon was induced by xylose even in the presence of glucose. To investigate regulation and physiologic functions we created several ClosTron and clean deletion mutants. We extended the study to another solventogenic species, *Clostridium saccharobutylicum* NCP 262 and identified the corresponding potential xylose degradation operons. We performed successful conjugation to knock out the genes via ClosTron. To achieve transformation we identified two operons encoding genes of presumed restriction-modification systems in *C. saccharobutylicum* NCP 262. The organisation of genes and sequence homology suggested they were both type I systems. We constructed two methylation plasmids, pJL-1 and pJL-2, to create an *in vivo* methylation system. Using them, we developed an efficient transformation method and tested various Gram-positive origins of replication for best fit for *C. saccharobutylicum*. We studied phenotypes of the constructed mutants of xylose degradation in rich and minimal media, containing different sugars as sole carbon sources. We focused on catabolite repression visible in several mutants and the variations in efficiency of consumption of various sugars.

IBP029
Bacterial proteins containing the DUF1521 domain as self-cleaving tools

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Our work focuses on the symbiosis between bacteria and legumes, in particular on the interaction of the soil bacterium *Bradyrhizobium japonicum* and its host plants, for example soybean. We have shown that *B. japonicum*

secretes proteins (Nops, nodulation outer proteins) upon induction by the plant signal genistein. We are interested in the type III-secreted effector protein NopE1, which exhibits autocleavage activity within two similar domains of unknown function (DUF1521). One DUF1521 domain is sufficient for autocleavage in the presence of calcium ions. Autocleavage is stable within a broad temperature and pH range [2]. Database searches indicate that the domain is also present in proteins of α-, β-, γ- and δ-Proteobacteria, e.g. the plant growth-promoting endophyte *Burkholderia phytofirmans* PsJN or the coral pathogen *Vibrio coralliilyticus* ATCC-BAA450. The putative protein Vic_001052 from *V. coralliilyticus* ATCC-BAA450 contains one DUF1521. Vic_001052 also shows self-cleavage in the presence of calcium. Based on these properties, we are developing an inducible and self-cleaving protein linker. In biotechnology, proteins are often expressed and purified as fusion proteins. In general, a protease is used to separate the protein of interest from the fusion partner. Our DUF1521 linker might provide an easy and low cost tool to liberate the protein from the fusion partner. For initial tests, the gene region encoding one DUF1521 domain of NopE1 and Vic_001052 was fused to different target genes and a *strep* tag. The fusions were expressed in *E. coli* and the recombinant proteins were purified by sepharose affinity chromatography. Tests indicate that auto-cleavage within NopE1and Vic_001052 is not influenced by the fused protein.

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IBP030

Novel and bioactive marine natural products from the marine-endophytic fungi *coniotherium cereale* and *auxarthron reticulatum*

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Endophytic marine-derived fungi are a prolific source of bioactive natural products. Investigation of the crude extract of the fungus *Coniothyrium cereale* provided a new series of phenalenone derivatives. Structurally most unusual and unprecedented are compounds with a lactam and imine functionality, as well as the heterodimeric compounds composed of a steroidal and a polyketide part. The structures of the compounds were established on the basis of extensive spectroscopic studies (^{1}H,^{13}C, HSQC, COSY, NOESY and HMBC as well as ^{1}H-^{15}N HMBC), mass spectral analysis (LC/MS and HRESIMS), UV and IR, in addition to X-ray crystalography. The analysis of absolute configuration was based on the chiroptical properties of the compounds. Biological investigations showed that several of these natural products have antimicrobial and cytotoxic activities, while others display inhibition of Human Leucocyte Elastase (HLE) in the lower micromolar range.
Investigation of the crude extract of the fungus Auxarthron reticulatum produced two promising alkaloids amauromine and methyl-penicinoline. The amauromine alkaloid showed a potent selective antagonistic activity toward CB_1 receptors (cannabinoid receptors type 1) with a Ki value 178 nM.

IBP031

More than just *Taq* polymerase - New glycoside hydrolases from *Thermus* for the application in biorefineries

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With the discovery of the first thermostable DNA polymerase from *Thermus aquaticus* in 1976 members of this genus became attractive candidates for scientific investigation. Since most of the species have been isolated from hydrothermal habitats with optimal growth temperatures of 70-80°C, *Thermus* is a promising source for thermostable industrial enzymes. During the last two decades several enzymes, such as proteases, catalases or DNA processing enzymes have been characterized from these thermophilic bacteria [1]. Although xylanolytic and cellulolytic *Thermus thermophilus*-strains were discovered [2, 3] comparatively few cellulases and so far no xylanase have been characterized. Especially for the application in „second generation biorefineries" where lignocellulosic material from agricultural or forestry residuals is used, thermostable enzymes from *Thermus* offer great potential to improve the efficiency of the production of sustainable biofuels.
Aiming at the discovery of new industrial relevant glycoside hydrolases several gene libraries from different *Thermus*-strains with distinct activity towards cellulose and xylan have been constructed and screened. Open Reading Frames coding for a new β-glucosidase and a new xylanase were identified. Both glycoside hydrolase encoding genes were successfully cloned and expressed in *E. coli* BL21 Star™ (DE3) and the recombinant proteins were purified. The characteristics of the enzymes (e.g. activity above 90°C, hydrolysis of cellobiose and xylan) offer a great potential for application in „second generation biorefineries". Further properties such as pH- and temperature-stabilities, as well as the influence of metal ions or detergents will be presented.

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IBP032

Search for alternative pathway(s) causing significant glycerol formation in a strain of *Saccharomyces cerevisiae* deleted in specific glycerol 3-phosphatase genes (*GPP1* and *GPP2*)

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The production of L-glycerol 3-phosphate (L-G3P) via microbial fermentation is interesting due to the compound's potential to replace the unstable dihydroxyacetone phosphate (DHAP) in one-pot enzymatic carbohydrate synthesis. A *S. cerevisiae* strain defective in the two genes encoding the two specific glycerol 3-phosphatase isoenzymes Gpp1 and Gpp2 strongly accumulates L-G3P and also produces a significant amount of glycerol (78% of the wild-type yield in g glycerol/g glucose) via an unknown alternative pathway [1]. In order to shed more light on the physiology of a *gpp1*Δ *gpp2*Δ double mutant, we launched a comparative microarray-based transcriptome analysis between this strain and the corresponding wild type. Surprisingly, *DAK2* encoding for dihydroxyacetone kinase showed by far the highest observed fold increase in the *gpp1*Δ *gpp2*Δ deletion strain (log2 ratio of 9.1). We deleted *DAK2* in the *gpp1*Δ *gpp2*Δ background but this did not result in a decreased glycerol production. In addition to *DAK2*, three other suspicious genes, *GCY1*, *DOG1* and *DOG2*, showed a higher expression level in the *gpp1*Δ *gpp2*Δ deletion strain (log2 ratios of 2.5, 2.8 and 1.2, respectively). The deletions of either *GCY1* or *DOG1/2* (*DOG1* and *DOG2* are located closely to each other and have been simultaneously deleted in one step) in the *gpp1*Δ *gpp2*Δ background did not show any significant decrease in glycerol yield. However, the quintuple deletion mutant *gpp1*Δ *gpp2*Δ *gcy1*Δ *dak2*Δ *dog1/2*Δ showed a 30% reduction in the glycerol yield as compared to the *gpp1*Δ *gpp2*Δ strain.

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IBP033

Application of membrane technology: An improvement of the biomass degradation in biogas plants?

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The University of Applied Sciences Nordhausen analyzed the effectivity of biogas synthesis by including a membrane to increase the degradation of organic substances in biogas plants. The investigations started in 9/2010 and are founded by the Federal Ministry of Food, Agriculture and Consumer Protection (BMELV) on the Agency for Renewable Resources (FNR).
The innovation of this method is the connection of selected tubular membranes with the 1 m³ fermenter of the experimental biogas plant. The installed membrane modules separate the contents of the fermenter in a solid (retentate) and a liquid phase (permeate). The solids are feed back into the fermenter while permeate is fermented separately. The process with maize silage as test substrate is based on the idea of decoupling the hydraulic retention time of readily biodegradable and of difficult as well as non-biodegradable fermenter ingredients on the one hand and a therefore resulting advanced degradation of organic matter on the other. The separated permeate contains fatty acids having in turn a significant residual gas

potential. In further investigations the project partner BTN Biotechnologie Nordhausen GmbH evaluates different fermenter designs for obtaining this gas potential of the separated permeat. With a successful operating separating membrane biogas plant operators have the opportunity to achieve higher gas yields based on the same substrate amounts or the same gas yield with less substrate amount, respectively.
The preliminary experimental results indicate further research, mainly in the field of membrane optimization and the impact of the membrane technology on the microbial diversity in the fermenter, respectively. In this regard also proteomic studies are necessary.

IBP034
Fermentation and spray formulation of an endophytic *Beauveria bassiana* strain

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The efficacy of many commercial insecticides has decreased in recent years due to increasing insect resistance. To protect plants against insects the entomopathogenic endophytic fungus *Beauveria bassiana* isolate ATP-04 can be utilized. To use an endophytic *B. bassiana* strain as a commercial biocontrol agent, the endophyte has to be mass-produced and formulated in such a fashion that it colonizes plants like oilseed rape and protects them from insect pests, just as transgenic plants do.
B. bassiana was raised in shake flask cultures to produce submerged conidiospores (SCS) which are reported to show a higher shelf life than mycelium and blastospores. In total, 23 technical culture media based on different carbon sources, minerals and technical yeast extracts were screened. It was found that in mineral media with 5% sugar beet molasses *B. bassiana* produced 0.1 x 10^{10} SCS/g sucrose in 170 h. By adding 50 g/L NaCl 48 h after inoculation the SCS yield was increased to 1.4 x 10^{10} SCS/g sucrose.
The scale-up to a 2 L stirred tank reactor was carried out at 25°C, 200-600 rpm and 1 vvm at pH 5.5. A total spore yield of 7.6 x 10^{10} spores/g sucrose respectively a SCS yield of 0.4 x 10^{10} SCS/g sucrose was obtained in mineral media with 5% molasses in 170 h. As in shake flask culture the yield of SCS was increased to 2.4 x 10^{10} SCS/g sucrose by the addition of NaCl.
After evaluation of three formulation strategies, namely encapsulation, seed treatment and spraying, it was decided that the spores from liquid fermentation should be formulated in a spray to support the colonization of oilseed rape plants with the fungus.
At first, potential spray formulations with 10^6 spores/mL combined with different surfactants, humectants, nutrients, sunscreens and other adjuvants were screened for their physiochemical properties to increase colonization of oilseed rape plantlets. Selected data on novel spray formulations such as contact angle, surface energy of oilseed rape leaves, Marangoni flow, droplet shape, spreading on leaf surface, drying, viability and germination of the fungus will be presented. Further experiments will include field trials and insect mortality tests.

IBP035
Production of secondary metabolites with endophytes isolated from a tropical tree

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Endophytes are microorganisms that live at least parts of their life cycle more or less asymptomatically in plants. The estimated high species diversity of endophytes and their adaption to various plant habitats presumes a rich and almost untapped source of new secondary metabolites for pharmaceutical or agricultural applications. Today a lot of interesting compounds from plants and trees are obtained via complex extractions in low concentrations. It is hypothesized that some of these pharmaceutical and agricultural compounds are produced by endophytes.
All parts of a tropical tree show an array of negative effects on insects including ovipositor deterrent, anti-feedant and other inhibitory activities. That is why we wanted to find out if this tree contains endophytic microorganisms and if these produce active secondary metabolites associated with the plant metabolism.
At first, plant material (seeds, leaves, stems) was collected. To eliminate epiphytic microorganisms, all the samples were surface-sterilized. The samples were immersed in 70% ethanol for 2 min and then sterilized with 5% sodium hypochlorite for 3 min and then rinsed again in 70 % ethanol for 2 min, before a final rinse in sterilized double-distilled water. Each sample was then surface dried under aseptic conditions. Segments of each sample were placed on SDA agar and were incubated for 7 days at 25°C. After isolation of 14 endophytes we cultivated the fungi and bacteria in complex liquid media for 14 days at 25°C. Terpenoid secondary metabolites that so far have been thought to be produced by the tree were detected in the culture broth of one bacterium and three fungi with HPLC-DAD.
Furthermore we will conduct basic research in cooperation with partners from genomics, transcriptomics and metabolomics, and apply the findings to the development of low-cost culture media and mass-production of the secondary metabolites in a 2 L stirred tank reactor.

IBP036
Optimization of glycoside hydrolase genes expression obtained by targeted metagenomics

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Amylomics is an acronym for the EU project "Amyloenzymes" captured from geothermal habitats e.g. hot spring and microbial mat in iceland by targeted Metagenomics. Total 10 research groups [1-10] from institute to company participate in the collaborative project. The aim is to develop new, robust enzymes for the starch and carbohydrate industries. The "Amyloenzyme" genes originate mainly from the thermophilic or mesophilic archaea. To overcome the low heterologous expression of these genes and the inclusion body formation, we explored solutions like the coexpression of chaperone (GroEL, GroES, DnaK, DnaJ or GrpE) and rare tRNA (argU/argW) genes, the fusion to MBP/NusA in *E.coli* strains like JM109, BL21 star(DE3)pLys and SoluBL21 and the use of *Sulfolobus* expression system [11]. We found that the combination of MBP-fusion and *E.coli* SoluBL21 resulted in high protein production and solubility. To obtain the native protein, Smt3 a small ubiquitin-like modifier protein and $(His)_6$ were inserted between MBP and target protein (MBP-Smt3-$(His)_6$-target), Smt3 can be processed specifically by Ulp1. After *in vitro* cleavage by Ulp1 the native target protein is released. It permitted the subsequent purification of target protein by conventional anion exchange chromatography or IMAC.

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IBP037
Engineering the citric/isocitric acid overproduction by the yeast *Yarrowia lipolytica*

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Question and Methods: Functionalized carboxylic acids are highly versatile chemical species with a wide range of applications (e.g. as co-polymers, building blocks, acidulants). Therefore they are of special interest as biotechnologically available targets. The yeast *Yarrowia lipolytica* secretes high amounts of various organic acids, like citric acid (CA) and isocitric acid (ICA) under several conditions of growth limitation from an excess of carbon source. Depending on the carbon source used, strains of *Y. lipolytica* produce a mixture of CA and ICA in a characteristic ratio. On carbohydrates and glycerol, wild-type strains show a CA/ICA ratio of 90:10, and on sunflower oil and n-alkanes of 60:40. To examine, whether this CA/ICA product ratio can be influenced, isocitrate lyase (*ICL1*), aconitase (*ACO1* or *ACO2*), NADP- (*IDP1*) or NAD-dependent (*IDH1* and *IDH2*) isocitrate dehydrogenases overexpressing strains were constructed containing multiple copies of these genes, respectively.
Results: In the *ICL1* overexpressing strains the part of ICA on the whole product (CA + ICA) decreased to 3-7% on all tested carbon sources [1]. In contrast, the *ACO1* [2] and interestingly also the *IDP1* overexpression resulted in a shift of the product pattern in direction of ICA. On carbohydrates the ICA proportion increased from 10-12% to 14-25%, on sunflower oil even from 35-55% to 65-72% of total acid produced. Therefore a combination of *ACO1* and *IDP1* overexpression is currently under investigation.

Conclusions: Thus, using wild-type or engineered *Y. lipolytica* strains the enantiomerically pure form of D-*threo*-isocitric acid, currently available as a speciality compound, can be produced now in large amounts and used as a building block for organic synthesis [3].

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IBP038

Screening of oleaginous microorganisms for the production of single cell oils as raw material for biofuels or fine chemicals

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Nowadays many consumer products like plastics or petrol are dependent on raw oil. The depletion of raw oil in the near future requires the use of renewable feedstock.
One possibility is the production of Single Cell Oil (SCO) by oleaginous microorganisms. Several yeasts, fungi, bacteria and microalgae belong to that group of lipid producing microorganisms and contain more than 20% intracellular fat per cellular dry weight. This fat is produced as carbon storage in the form of triacylglycerids when a carbon source is in excess, but other limitations like depletion of nitrogen source prevent further cell division. Depending on culturing conditions the percentage of SCO can be increased up to 70% in several strains.
For industrial applications it is important to consider not only the amount of produced SCO, but also the quality characteristics like the fatty acid profile. High value fatty acids like Ω -fatty acids for pharmaceutical applications could be economic in low amounts while low value fatty acids like oleic acid for biodiesel production has to be produced in high amounts to be worthwhile.
With efficient screening methods we identified three new oleaginous microorganisms with interesting fatty acid profiles which are adequate for industrial applications.

IBP039

Metabolic engineering of *Pseudomonas putida* KT2440 for the production of compounds derived from the shikimic acid pathway

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The shikimic acid pathway intermediate chorismic acid is the branching point to the biosynthesis of the aromatic amino acids and other aromatic compounds. We propose an engineered biosynthetic pathway for aromatic substances based on simple sugars like glucose, using *Pseudomonas putida* KT2440 as the host of choice. *Pseudomonas putida* KT2440 is the best characterized strain of the saprophytic Pseudomonads and has been certified as a biosafety host for cloning of foreign genes; no virulence factors were detected according to genomic analysis [1]. We intended to increase the flux towards chorismic acid by introduction and expression of orthologous genes from *E. coli* to add to inherent enzyme functions of *P. putida*. As an example, the *aroFBL* genes, encoding flux-limiting enzymatic reactions in the shikimic acid pathway of *E. coli* [2,3], were chromosomally integrated into *P. putida* strains. In addition, reactions competing for chorismic acid were removed by genetic knock-outs. The deletion of genes *trp*E and *phe*A led to double auxotroph (L-tryptophan, L-phenylalanine) strains [4]. To implement the chromosomal changes, a method for markerless gene insertion and subsequent deletion based on homologous recombination and counterselection with the antimetabolite 5-fluorouracil in an *upp* mutant strain was used [5].
These deletion mutant strains excreted 6 mM of chorismic acid (from 50 mM glucose) into the supernatant of mineral salt medium. Addition of the *aroFBL* genes from *E. coli* enhanced chorismic acid production to 7 mM. In contrast, the wild type strain did not excrete chorismic acid. Based on these chorismate-producing strains with chromosomally integrated genes, further genes were added on expression plasmids which were newly created for use in *P. putida*.
Thus, it became possible to redirect the aromatic amino acid pathway to the production of chorismate-derived products. Examples will be given.

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IBP040

2-Ketoisocaproate Production with *Corynebacterium glutamicum*

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Corynebacterium glutamicum is a non-pathogenic, Gram-positive organism that grows on a variety of substrates and is used for the production of amino acids, e.g.
L-glutamate, L-lysine and L-valine, as well as organic acids, e.g. lactic and succinic acid. The aim of the present work was to engineer *C. glutamicum* to produce 2-ketoisocaproate (KIC; 2-keto-4-metyl-pentanoate). This ketoacid is used as a therapeutic agent and a KIC-producing organism may serve as a platform for products deriving from this 2-ketoacid, e.g., 3-methyl-1-butanol. We engineered the wild type of *C. glutamicum* for the growth-coupled production of KIC from glucose by deletion of *ltbR and ilvE* genes, encoding the L-leucine and tryptophan biosynthesis regulator Lrp and transaminase B, respectively, and additional overexpression of the *ilvBNCD* genes, encoding the L-valine biosynthetic enzymes acetohydroxyacid synthase, acetohydroxyacid isomeroreductase, and dihydroxyacid dehydratase. KIC production was further improved by deletion of the methylcitrate synthase genes *prpC1* and *prpC2* and decreasing citrate synthase activity by exchange of the native promoter of the citrate synthase gene *gltA*. In shake flask fermentations under L-leucine limitation, the newly constructed strains produced up to
30 mM (3.9 g l^{-1}) KIC and showed a product yield of about 0.26 mol per mol of glucose in the overall production phase. The concomitant formation of significant amounts of 2-ketoisovalerate (KIV) indicates a limitation of KIC production in the pathway leading from KIV to KIC.

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IBP041

Bioplastic production in the microalgae *Phaeodactylum tricornutum* - an electron microscopical survey

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Microalgae like the diatom *Phaeodactylum tricornutum* represent an important lipid and silicate source. Moreover, their phototrophic lifestyle and a CO_2-neutral cultivation with low costs makes them interesting for various biotechnical applications. Recent studies could show that they are also suitable for the biotechnical expression of macromolecules and recombinant proteins. Their high growth rate together with the advantages of eukaryotic expression systems like post-transcriptional and post-translational modifications are of great benefit for biotechnological applications. Recently, it was shown that a monoclonal human IgG antibody and the respective antigen can be produced in *P. tricornutum*. Interestingly, the antibodies were fully-assembled and functional and could also be secreted into the culture medium with high efficiency [1]. Moreover, *P. tricornutum* was used as a cellular production factory for the bioplastic poly-3-hydroxybutyrate (PHB) which resulted in levels of about 10% of algal dry weight [2].

A very important point in these experiments represents the localization of the proteins and bioproducts on the one hand via fluorescent light microscopy, and on the other hand by application of cryo preparation methods like high-pressure freezing and freeze-substitution for subsequent electron microscopy [3,4].

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IBP042

Raw starch utilization and L-lysine formation by a recombinant *Coryne-bacterium glutamicum* strain

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Corynebacterium glutamicum is well known for the industrial production of amino acids, such as L-lysine. This Gram-positive soil bacterium grows aerobically on a variety of mono- and disaccharides but not on high-molecular-weight carbohydrates, such as maltodextrins or starch. In industrial amino acid fermentations, the costs of substrates are important variables, thus the manufacturers target to use the complex raw material more efficiently to avoid the cost-intensive hydrolysis of starch. Therefore, we constructed the L-lysine-producer *C. glutamicum* DM1729(pAMY) synthesizing and secreting the alpha-amylase from *Streptomyces griseus* by transformation of an expression vector carrying the alpha-amylase gene. Although some high-molecular-weight degradation products remain in the culture broth, this recombinant strain efficiently used starch from potato, corn, wheat and rice as carbon and energy substrates for growth and also for L-lysine production. The yields and specific productivities of *C. glutamicum* DM1729(pAMY) were different depending on the different starches and depending on whether raw or boiled (solved) was employed. Electron microscopic analysis of the different starch grains revealed differences in morphology and size. In addition, there were obvious differences between raw and boiled starch grains. However, the recombinant strain showed about the same L-lysine yields with raw and solved corn, wheat, and rice starch as carbon sources compared to *C. glutamicum* DM1729 grown with a comparable amount of glucose. Taken together, we could show that the recombinant strain *C. glutamicum* DM1729(pAMY) is able to use different raw and solved starches as energy and carbon source for growth and L-lysine production.

IBP043

Novel thermoactive and -stable cellulases for improved lignocellulosic biomass degradation

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In the last years strategies have been developed to utilize mostly starch-based biomass as a resource for the production of energy and chemicals. To avoid competition with food, future trend biorefineries using lignocellulosic biomass have been launched. Lignocellulose as wheat straw is a challenging substrate due to the compact, often crystalline structure of cellulose-, hemicellulose- and lignin-polymers. The cellulosic and hemicellulosic fractions resulting from hydrothermal decomposition are degraded enzymatically to fermentable sugars. The finding of novel thermoactive and thermostable enzymes will contribute to the development of efficient processes for the bioconversion of cellulosic biomass.
Metagenomic libraries were constructed from environmental samples (compost and hot springs from the Azores) and screened at 70°C for the presence of cellulase encoding genes. The detection of endoglucanase, cellobiohydrolase and β-glucosidase activities was achieved by colorimetric assays. The activity-based screening revealed two genes encoding putative β-glucosidases (*bgl*1 and *bgl*2). Identities of 80% to uncharacterized bacterial and less than 55% to uncharacterized archaeal enzymes were obtained for the amino acid sequence of the two β-glucosidases. Both enzymes are active at high temperatures and Bgl2 degrades cellobiose and cellotriose. Both enzymes show optimal activity at 90°C with a pH-range from 5 to 9.
Hence, both enzymes are attractive candidates for industrial applications and especially Bgl2 for cellulose degradation after hydrothermal treatment and consequently it can contribute in the development of a sustainable biorefinery of the second generation.

IBP044

Amino acid production from rice straw hydrolysate by recombinant pentose-utilizing *Corynebacterium glutamicum*

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The resources of fossil oil are becoming limiting. Lignocellulosic biomass is a cheap non-food carbon source available from agricultural wastes, which may represent viable alternative carbon sources for biotechnology.
Corynebacterium glutamicum wild type lacks the ability to utilize the pentose fractions of lignocellulosic hydrolysates, but it is known that recombinants expressing the *araBAD* operon from *Escherichia coli* are able to grow with the pentose arabinose and recombinants expressing the *xylA* gene from *E. coli* are able to grow with xylose as sole carbon sources. Recombinant pentose-utilizing strains derived from *C. glutamicum* wild type or from the L-lysine producing *C. glutamicum* strain DM1729 utilized arabinose and/or xylose when these were added as pure chemicals to glucose-based minimal medium or when they were present in acid hydrolysate of rice straw (1, 2).
Comparison of growth with mixtures of pure chemicals to growth with acid hydrolysate growth revealed delayed and slower amino acid production and sugar consumption with acid hydrolysates than with blends of pure arabinose, xylose, and glucose.
To improve xylose utilization, different xylose isomerase genes and xylulokinase genes were tested in *C. glutamicum*. Combining expression of the xylose isomerase gene from *Xanthomonas campestris* and of the *C. glutamicum* xylulokinase gene resulted in fast growth with xylose. The growth rate doubled as compared to the previously described strain solely expressing the *E. coli* xylose isomerase gene. Accordingly, the lysine productivity of a lysine producing strain was increased in media containing rice straw hydrolysate as carbon source (3).
This study clearly revealed the potential *C. glutamicum*, workhorse of million-ton-scale amino acid production, for utilization of second generation feedstocks.

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IBP045

Microbial poly(3-hydroxypropionate)-production in *Shimwellia blattae*

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In recent years glycerol has become an attractive carbon source due to its occurrence as a byproduct in biodiesel production. Glycerol is formed by transesterification of fats or vegetable oils in presence of methanol to methylacylesters. The tremendous growth of the biodiesel industry has generated a glycerol surplus resulting in a sharp decrease of glycerol prices. A potential use of glycerol in biotechnology is the microbial synthesis of polyhydroxyalkanoates (PHAs), which are bacterial storage compounds for carbon and energy. PHAs are synthesized and deposited as intracellular granules in many bacteria. They are biodegradable, insoluble in water, non-toxic, biocompatible, piezoelectric, thermoplastic and/or elastomeric. These features make PHAs suitable for several applications in medicine, pharmacy, agriculture, packaging- and food industry, as raw material for production of enantiomerically pure chemicals and for the production of paints. We have developed a process for the conversion of (crude) glycerol to the thermoplast poly(3-hydroxypropionate)[1] in the natural 1,3-propanediol producer *Shimwellia blattae* ATCC33430. For this we expressed the genes for (i) 1,3-propanediol dehydrogenase DhaT, (ii) aldehyde dehydrogenase AldD (both from *Pseudomonas putida* KT 2442), (iii) propionate CoA-transferase Pct from *Clostridium propionicum* X2 and (iv) PHA synthase PhaC1 from *Ralstonia eutropha* H16 heterologously *in vivo*. In a two-step fermentation process the recombinant strain was able to accumulate up to 10 % (wt/wt CDW) of poly(3HP). The polymer, which was isolated through treatment

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IBP046

Heterologous expression/secretion of a bacterial aspartase and transaminase in the yeast *Yarrowia lipolytica*

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Heterologous gene expression and secretion of gene products by *Yarrowia lipolytica* was mainly focused on proteins of higher organisms. To evaluate the ability of *Y. lipolytica* to produce bacterial proteins the expression and secretion of a bacterial aspartase and a transaminase was investigated. The secretion is driven via different parts of the prevalent used secretion signal sequence of the extracellular alkaline protease XPR2. For the construction of a production strain of *Yarrowia lipolytica* at first the genes for its extracellular proteases were deleted. Prior to integrative transformation the codon bias of the heterologous genes were optimized for *Yarrowia lipolytica*. The characterization of the recombinant *Y. lipolytica* strains was focused on the effectiveness of the respective secretion signal in enzyme production. This was investigated in dependence of the constitutive expression of the heterologous genes by the pTEF1alpha promoter in strains which have the expression cassettes integrated as single copy or as multi-copies.

IBP047

End-to-end gene fusions and their impact on the production of multifunctional biomass degrading enzymes

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Cellulose and hemicellulose are complex polymeric substrates comprising 70-80% of lignocellulose of plant cells. The bioconversion of these complex substrates has proven to be of great importance industrially, ranging from textile industry to food processing and biofuel production. A number of bacterial enzymes work synergistically to efficiently digest polysaccharides, such as cellulose and hemicellulose. Xylanases hydrolyze β-1,4 glycosidic linkages of hemicellulose. Cellulases catalyze random cleavage of the cellulose chain, and β-glucosidases hydrolyze cellobiose to glucose. Extremophilic microorganisms are suitabel candidates for industrial enzymes due tot he fact that they produce highly thermostable enzymes. However, high enzyme production cost is still a major issue. Bifunctional enzymes harboring dual enzymatic activities are cost effective, resulting in highly synergistic and active constructs. Processes that occur in nature can be mimicked owing to different, advanced, molecular and genetic techniques (1).
In this study we report the construction of bifunctional variants generated for the optimal degradation of polysaccharides. A cellulase-xylanase variant with an 8 amino acid linker situated between the 2 genes, showed activity when xylanase was fused both downstream and upstream of cellulase, with optimal activity at 80°C over 4 hours. Cellulase- β-glucosidase constructs with varying linker lengths composed of the same amino acid stretches were also assembled in order to study the effect of linker length and the total construct stability and activity. Detailed biochemical properties including temperature and thermostability optima as well as synergestic effects will be presented.

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IBP048

Reduced byproduct formation by overexpression of the genes encoding the trehalose uptake system TusEFGK$_2$ in fed-batch cultivations of *Coryne-bacterium glutamicum*

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Corynebacterium glutamicum is a Gram-positive soil bacterium used for the large-scale industrial production of the amino acids L-glutamate and L-lysine. We recently described growth of *C. glutamicum* with the disaccharide trehalose and identified the trehalose uptake system. This trehalose-specific ABC-transporter of *C. glutamicum* is formed by the ATPase TusK, the trehalose binding protein TusE, and the two permeases TusG and TusF. This highly specific uptake system (K_M value 0.16 μM) transports trehalose with a maximum rate of 2.5 nmol × mg cdw^{-1} × min^{-1}.
Besides its role as a source of carbon and energy, trehalose is a major building block of the mycolic acids, which form an outer permeability barrier in *Corynebacterianeae*. The trehalose uptake system is responsible for the reuptake of trehalose produced in the last, extracellular step of the trehalose dimycolate biosynthesis. Consequently, trehalose does not accumulate in shaking flask cultures of *C. glutamicum*.
In contradiction to the presence of a high affinity trehalose uptake system, accumulation of trehalose as a byproduct in the culture broth of *C. glutamicum* amino acid fermentations has been described (1). We here addressed the reason for this accumulation of trehalose and therefore performed high-cell-density fed-batch cultivations of the L-lysine producing *C. glutamicum* DM1729. In difference to shake flask experiments with *C. glutamicum* DM1729, we observed final titers of up to 8 mM of trehalose in the supernatants of fed-batch fermentations. Analysis of the expression of the *tus* genes in the course of fed-batch fermentations revealed the absence of *tusK*, *tusE*, and *tusGF* transcripts in the stationary growth phase. Accordingly, no activity of the trehalose uptake system was measured in cells from the stationary growth phase. The lack of *tus* expression in the stationary phase of fed-batch fermentations was overcome by episomal overexpression of the *tus* gene cluster under the control of an IPTG-inducible promoter, improving the activity of the trehalose uptake system, thus eliminating the accumulation of trehalose and increasing the L-lysine yield. Hence the trehalose uptake system is an interesting target for optimization of industrially applied *C. glutamicum* strains.

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IBP049

Characterization of two distinct Fructose 1,6-bisphosphate aldolases from *Bacillus methanolicus* MGA3 and their physiological role

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The Gram-positiv methylotrophic thermotolerant *Bacillus methanolicus* is able to grow with methanol as well as with glucose or mannitol a sole carbon and energy source. Fructose 1,6-biphosphate aldolase (FBA), a key enzyme of glycolysis and gluconeogenesis is encoded in the genome of *B. methanolicus* by two putative *fba* genes, the chromosomally located *fba*^{C} and *fba*^{P} on the naturally occurring plasmid pBM19. Their amino acid sequences share 75% sequence identity and suggest a classification as class II aldolases. Both enzymes were purified from recombinant *E. coli* and were found to be active as homotetramers. Both enzymes were activated by either manganese or cobalt ions and inhibited by ADP, ATP and EDTA. The kinetic parameters allow to distinguish the chromosomally encoded FBA^C from the plasmid encoded FBA^P. FBA^C showed higher affinity towards fructose 1,6-bisphosphate (K_M 0.16 mM vs. 2 mM for fructose 1,6-bisphosphate) as well as a higher glycolytic catalytic efficiency than FBA^P. On the other hand, FBA^P exhibited higher affinity to triose phosphates than FBA^C (K_M 0.25 mM and 0.58 mM vs. K_M 0.6 mM and 1 mM for glyceraldehyde 3-phosphate and dihydroxyacetone phosphate, respectively) as well as a higher catalytic efficiency in the gluconeogenetic direction. Complementation of the aldolase-negative mutant *C. glutamicum* Δ*fda* with *fba*^{C} and *fba*^{P} from *B. methanolicus* supported this classification.
According to the determination of the substrate affinity, we propose that FBA^C has a higher catalytic efficiency and therefore seems to be the major aldolase in glycolysis whereas FBA^P is the major aldolase in gluconeogenesis at low concentrations.

IBP050

Different signal peptides improve lipase secretion by *Burkholderia glumae*

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Industrial relevant products are often obtained by enzyme-catalyzed reactions[1]. Among industrial enzymes, lipases have extensively been used for manufacturing a variety of products, e.g. in the food and pharmaceutical industry. A lipase originating from *Burkholderia glumae* PG1 is used for the production of enantiopure alcohols and amines by BASF AG[2]. Over the years, the lipase production strain was subjected to many rounds of classical mutagenesis thereby improving the yield of the target enzyme.
The mechanism of lipase secretion by *B. glumae* has been investigated in detail. The lipase LipA is first transported by the Sec system across the inner membrane into the periplasm and subsequently passes the outer membrane *via* the general secretion pathway (GSP). LipA possesses an N-terminal signal peptide consisting of 39 amino acids which serves as a recognition signal for the Sec system. Although the overall composition of these signal peptides is similar in all Sec-translocated proteins, it is known that the secretion efficiency can be modulated by fusing different signal peptides to a given target protein[3]. Improved secretion efficiency results in an increased enzyme yield and thus lowers costs for the industrial process.
We have constructed a signal peptide library consisting of 72 signal peptides obtained from the Gram-negative bacteria *Escherichia coli*, *Pseudomonas putida* and *P. aeruginosa*. Signal peptides were cloned upstream of the *lipA* gene and subsequently transformed into the *B. glumae* PG1 expression host. About 200 clones (3-fold oversampling) were cultivated as duplicates in microtiter-plates and the culture supernatants were analyzed with respect to lipase activity. Our results showed that each signal peptide had a significant influence on lipase activity recovered from the culture supernatant with the best performing variants showing up to 1.4 fold increased activity. The observed effect was reproducible also upon cultivation of the best performing signal peptide-lipase fusions in shaking flasks with a culture volume of 20 ml. Further experiments will show whether the change in the signal sequence also has an influence on the transcript stability and on the transport across the outer membrane.

1: Drepper *et al*. (2006). Biotechnol. J. 1:777-786.
2: Braatz *et al*. (1993). WO 9300924 A1 (23.06.92). Chem. Abstr. 118:175893.
3: Degering *et al*. (2010). Appl. Environ. Microbiol. 76(19):6370-6.

IBP051

Autoaggregation of Pseudomonas putida KT2440 as a stress response towards solvent exposure is dependent on LapA and regulated by c-di-GMP

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As a potential next generation biofuel and a solvent used in various commercial products, n-butanol represents a very attractive compound for biotechnological approaches. The microbial production of chemicals offers great economic and ecological potential, but also possesses intrinsic challenges, especially when it comes to hydrophobic substances.
We found that for *Pseudomonas putida* KT2440 concentrations greater than 100 mM of butanol were growth inhibitory and caused a steady decrease in cell survival during long-term incubations in a concentration dependent manner. The level of persistence varied between cells from exponential- and stationary phase, with the latter being more resistant. Further, survival of cells was correlated with growth permitting conditions, as the absence of glucose or oxygen and the presence of the uncoupler CCCP significantly increased the sensitivity of the cells towards the toxic effects of the solvent.
When exposed to sub-lethal concentrations of butanol, the majority of the cells responded with the formation of cell-cell aggregates and increased biofilm formation. This active stress response correlated with the butanol concentration used and was found to strictly depend on the availability of energy for the cell, elevated intracellular c-di-GMP levels, and the presence of the surface adhesin LapA. Interestingly, the survival of cells in the presence of butanol did not correlate with the ability to form aggregates. However, when challenged with additional energy stress, aggregate formation increased the fitness of the cells significantly.
From these data we conclude, that *P. putida* KT2440 responds to butanol stress with phenotypic diversification of cells, representing a new example for common adaptation strategy of microorganisms for the survival varying environmental conditions.

IBP052

Identification of a Caldariomyces fumago Mutant Secreting an Inactive Form of Chloroperoxidase Lacking the Heme Group and N-glycans

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By mutant colony screening of Caldariomyces fumago a mutant was isolated which was slightly greenish on fructose minimal medium and grew slower in comparison to the wild type. The supernatant samples lacked the Soret band typical for the heme group of the CPO and nearly no CPO activity was detected. SDS-PAGE analysis of
mutant culture supernatant samples showed production of a 38 kDa protein while wild type samples contain the 42 kDa CPO protein. Protein identification using nanoLC-ESI-MS/MS was performed and based on three peptides the protein in the mutant culture was identified as CPO. No differences in the CPO gene sequences of wild type and mutant were found indicating a post-translational defect in protein maturation. Deglycosylation experiments using CPO from wild type and mutant were carried out. After removing N-linked oligosaccharides from wild type CPO a protein band at 38 kDa was detected. Our results reveal that the mutant protein lacks the heme group as well as the N-glycans and suggest a causal link between these two CPO maturation phenotypes.

MMIV001

Spying on the lives of marine microbes: The power of direct observation

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At a time when microbial ecology is largely traveling along genomic roads, we cannot forget that the functions and services of microbes depend greatly on their behaviors, encounters, and interactions with their environment. New technologies, including microfluidics, high-speed video microscopy and image analysis, provide a powerful opportunity to spy on the lives of microbes, directly observing their behaviors at the spatiotemporal resolution most relevant to their ecology. I will illustrate this 'natural history approach to microbial ecology' by focusing on marine bacteria, unveiling striking adaptations in their motility and chemotaxis and describing how these are connected to their incredibly dynamic, gradient-rich microenvironments.
Specifically, I will present (i) nanometer-resolution imaging at thousand frames per second to demonstrate that marine bacteria have a unique mode of swimming, vastly different from classic *E. coli* motility; and (ii) microfluidic experiments that capture the dramatic chemotactic abilities of bacterial pathogens towards the roiling surface of coral hosts. Through these examples, I aim to convince you that direct visualization can foster a new layer of understanding in microbial ecology.

MMIV002

Major role of photoheterotrophic and CO oxidizing *Roseobacter* RCA popu-lation in the ocean

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The *Roseobacter* clade is one of the most prominent phylogenetic lineages of marine Alphaproteobacteria. Its largest cluster, the *Roseobacter* Clade Affiliated (RCA) cluster, is most abundant in temperate and (sub)polar oceans, constituting up to 20% and 35% of total bacterioplankton, respectively. Based on the analysis of the sequenced and closed genome of an RCA isolate, Cand. *P. temperata*, retrieved from the southern North Sea, we were able to reconstruct its genome to 85% at the GOS stations of the western temperate Atlantic. In these samples this genome constituted 0.7 to 6.5% of total reads. Further, the genome was reconstructed to 78-85% in metagenomic libraries from the English Channel and a Norwegian Fjord, demonstrating its Atlantic-wide distribution. The genome size is 3.29 Mbp, the second smallest of all genomes of the *Roseobacter* clade. It carries 1

prophage and 10 genomic islands relative to typical other roseobacter organisms. The entire photosynthetic operon is present as well as both carbon monoxide (CO) oxidation gene clusters (coxI and II), enabling Cand. *P. temperata* to generate energy via aerobic anoxygenic photosynthesis as well as by carboxidovory, complementary to respiration. In a phytoplankton spring bloom in the southern North Sea, in which the RCA cluster constituted 5% of total bacterioplankton, we were able to reconstruct the genome of Cand. *P. temperata* to 87% in the metagenome and to 85% in the metatranscriptome. The metatranscriptome analysis revealed that this population simultaneously generated energy via aerobic anoxygenic photosynthesis and CO oxidation, in addition to aerobic respiration. This is the first report of the active participation of an aerobic anoxygenic and CO oxidizing photolithoheterotrophic bacterium in ocean biogeochemistry. It reveals that this organism of the RCA cluster exhibits an exceptionally versatile adaptation to the nutrient-deprived pelagic environment.

MMIV003

Distribution of clusters of the *Roseobacter* clade in global oceans

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The *Roseobacter* clade of *Alphaproteobacteria* is an important component of the marine bacterioplankton. Members of this clade can constitute large proportions of total *Bacteria*. However, most of the *Roseobacter* clusters identified in pelagic environments consist predominantly of uncultured phylotypes and only scarce information exists on the simultaneous occurrence of distinct subclusters.
In order to elucidate the occurrence of major pelagic subclusters of the *Roseobacter* clade, we analysed samples collected during different cruises around the world for the presence of the following subclusters: RCA, NAC11-6, NAC11-7 and CHAB-I-5. The spatial distribution of these clusters was investigated by DNA extraction from the free living bacterial fractions of surface waters and subsequent PCR with cluster-specific primers. Furthermore we examined representative depth profiles to trace these subclusters in different water masses along a transect across the Atlantic Ocean, from Antarctic waters to the Gulf of Biscay. To better understand the biogeochemical and environmental conditions for the occurrence of these clusters parameters such as inorganic nutrients (phosphate, nitrate, nitrite), dissolved amino acids, chlorophyll *a*, bacterial cell numbers, biomass production and turnover rates of amino acids and glucose were also assessed.
The RCA, NAC11-6 and NAC11-7 clusters were detected only in temperate to polar regions as well as in cold water masses like upwelling systems. Cluster CHAB-I-5 showed less distinct pattern.

MMIV004

Regulation of anaerobic respiratory pathways in *Dinoroseobacter shibae*

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Denitrification is part of the global nitrogen cycle and an important mechanism of energy generation under anaerobic conditions. *Dinoroseobacter shibae*, a representative of the globally abundant marine *Roseobacter* clade, is used as a model organism to study the transcriptional response to oxygen depletion in the presence of nitrate. Its annotated 4.4 Mb genome sequence revealed clustered genes which are involved in anaerobic respiratory energy metabolism with nitrate as an alternative electron acceptor. *D. shibae* features *nir*, *nor* and *nos* operons next to the *nap* operon. Interestingly, *D. shibae* possess the periplasmic nitrate reductase Nap instead of the membrane bound Nar as described for other denitrifiers. Usually, anaerobic and N-oxide dependent expression of the denitrification genes is often controlled be regulators of the Crp/Fnr family. An unusual high number of Crp/Fnr-like regulators were annotated. Beside a FnrL-homologue with a $[4Fe\text{-}4S]^{2+}$-cluster, six Dnr-like regulators were found. Two of those genes, encoding DnrD and DnrE are directly located between the *nor*- and *nos*-operon. We are interested in adaptive gene regulatory networks of D. shibae coordinating the shift from aerobic to anaerobic growth conditions. Therefore, we used a continuous cultivation of *D. shibae* in a chemostat combined with time resolved RNA microarray and proteome analyses. Transcriptome analyses revealed distinct patterns of gene expression in response to oxygen limitation. The change from aerobic to anaerobic growth showed a sequential induction of gene clusters encoding the denitrification machinery. Genes encoding the Fnr/Crp-like regulators showed different expression levels over time. The proteome dataset complemented the transcriptome analyses.
The transcriptome profile of aΔ *dnrF* knock-out mutant indicated a cascade of Dnr-like transcription factors involved in the regulation of the denitrification machinery.
We predicted a regulatory network for the anaerobic respiratory pathway in *D. shibae*. To confirm the regulon, heterologously produced DnrD, DnrE and DnrF were used for the identification of specific target promotors via DNA binding studies.

MMIV005

Diatoms - widespread dissimilatory nitrate reducers with overlooked impact on the marine nitrogen cycle

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The world's marine ecosystems teem with diatoms - the key group of the eukaryotic phytoplankton that is found throughout the world's oceans from polar to tropical latitudes, from massive phytoplankton blooms to vast abundances in coastal sediments. Diatoms are responsible for 40% of the marine primary production and are thus extremely important players in the carbon cycle. However, diatoms can also survive for decades buried deep within the dark, oxygen-depleted sediment layers at the seafloor, where neither photosynthesis nor aerobic respiration is possible. The survival mechanism under these non-phototrophic conditions has long remained enigmatic. Only recently we discovered a correlation between the dark survival potential of marine diatoms and their ability to accumulate nitrate intracellularly. 15N-labelling experiments proved that the benthic diatom *Amphora coffeaeformis* consumes the stored nitrate and that the consumption was accompanied by the production and release of 15N-labeled ammonium, indicating Dissimilatory Nitrate Reduction to Ammonium (DNRA). Similar results are now also available for the ubiquitous and highly abundant pelagic diatom *Thalassiosira weissflogii*. These are the first and so far only findings of dissimilatory nitrate reduction by any kind of unicellular phototrophic eukaryotes. Given the vast diversity and abundance of diatoms in various aquatic ecosystems, the storage and dissimilatory use of nitrate by diatoms may have significant implications for nitrogen cycling in oxygen-deficient water bodies and coastal sediments.

MMIV006

Co-assimilation of organic carbon by Nitrosopumilus maritimus suggests utilization of oceanic DOC by marine ammonia-oxidizing archaea

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Marine ammonia-oxidizing archaea (AOA) represent one of the largest microbial groups in the ocean. Their ubiquity and high relative abundance are indicative for their important role in marine biogeochemical cycles. The only pure culture of a marine AOA available to date, *Nitrosopumilus maritimus*, grows chemolithoautotrophically by oxidizing ammonia to nitrite and by assimilating inorganic carbon via a hydroxylpropionate/hydroxybutyrate pathway. Here, we report the capacity of *N. maritimus* to utilize various organic substrates as additional carbon sources for biosynthesis. Amendment of cultures with low concentrations (μM range) of certain intermediates of the central carbon assimilation pathways increased the specific cell yield per unit of ammonia oxidized, and reduced the maximum doubling time to less than 20 hours. Nevertheless, supply of inorganic carbon remained essential for growth. Cultivation experiments of *N. maritimus* with isotopically labeled bicarbonate (^{13}C) and glyoxylic acid (^{14}C) in combination with nanoSIMS analysis showed that *N. maritimus* formed just a minor part of its biomass from added organic carbon, the major fraction was still derived from inorganic carbon. In addition, in the presence of glyoxylic acid and oxaloacetic acid, cultures gradually fed ammonia reached a maximum cell density almost ten times higher than

achieved under strictly autotrophic conditions, indicating a yet unknown regulartory function in controlling the population size. The co-assimilation of organic carbon enables *N. maritimus* to reduce the demand of ATP as well as of adequate reducing equivalence for anabolic reactions. In addition to their reported high affinity to ammonia, a contribution of DOC to higher specific cell yield and growth rate might offer an ecological advantage in oligotrophic habitats. Our results further support previous findings that a portion of thaumarchaeal biomass in the ocean originates from the assimilation of dissolved organic carbon.

MMIV007

Dilution cultivation yielded novel psychrophilic marine bacteria, repre-senttatives of the phytoplankton decomposing community

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One of the major challenges in modern marine microbiology is establishing pure cultures of representative species that are characterised by high abundance and ecological relevance. The ecological roles of these microorganisms are increasingly explored by metagenomic approaches. However, the sparse knowledge on physiological characteristics often confounds the interpretation of such data. Strains in cultivation are increasingly in demand to understand the physiological activities in the oceans.
We isolated dominating microorganisms from the waters near the island Helgoland in the German Bight during a phytoplankton bloom that recurs annually in spring. Such phytoplankton blooms cause a pronounced response of the bacterioplankton community resulting in the remineralisation of half of the net primary production in the "microbial loop". An aerobic artificial seawater medium was devised reflecting the natural nutrient (C, N, P) concentrations that enabled a cell density of $5x10^6$ cells per millilitre. Furthermore, dilution cultures were inoculated with one nanolitre seawater directly after sampling, incubated for 3 months and screened for growth by flow cytometry.
Cultured marine bacteria were affiliated with the genera *Formosa* and *Polaribacter* (*Flavobacteriaceae*), *Reinekea* (*Gammaproteobacteria*), and the *Roseobacter* clade affiliated (RCA) lineage (*Alphaproteobacteria*). These phylotypes constitutes up to 50% of the total bacterioplankton cells in the seawater of Helgoland shortly after the peak of the spring phytoplankton bloom in 2009.
Draft genomes of the *Flavobacteriaceae* isolates were studied for pathways and gene cluster of polymer degradation (e.g. cellulose, chitin and alginate) and compared to *in situ* expressed enzymes as detected by metatransciptomics and metaproteomics. The physiological capacities were tested by growth of the isolates on different carbon sources and polymers. These studies identified factors that define the ecological niches of relevant marine microorganisms involved in the phytoplankton decomposition.

MMIV008

Bacterial distribution on marine now aggregates and its implication on the marine carbon cycle

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The area off Cape Blanc (NW Africa) is the second most productive of the Eastern Boundary Upwelling Systems in the world. Here the upwelling of South Atlantic Central Water leads to phytoplankton blooms and thereby to an enhanced production of marine snow aggregates. These aggregates sink to deeper water layers and finally sediment on the sea floor - a process known as the biological carbon pump. During sinking, a diverse microbial community on the aggregates degrades various carbon compounds, a process called the microbial loop. Earlier studies suggested that the microbial community changes with depth during the sinking process. In addition, a 3.5 fold higher respiration rate was found near surface waters at 15°C compared to lower depths at 4°C, suggesting a temperature dependence of the microbial activity. We compared the microbial community structure on marine snow aggregates, which were reconstituted in roller tanks incubation from water samples of 65 m and 400 m depth. First results with fluorescence *in situ* hybridization (FISH) showed that the aggregates, especially the transparent exopolymers (TEP), were dominated (up to 90%) by *Gammaproteobacteria* of the genus *Alteromonas* at both depths, suggesting a stable microbial community with depth. Since a bottle effect could not be excluded in the 20h - long roller tank incubations, drifter traps samples from of 100 m and 400 m depth were used to investigate the microbial community on aggregates from non-incubated samples. The gentle sampling method preserved the native three dimensional structure of the aggregates and allowed for FISH investigations of the distribution of the microorganisms on the aggregates. A clear dominance of *Alteromonas* could not be reconciled on the aggregates but instead a mixed community of mainly *Bacteroidetes* and *Gammaproteobacteria* was detected. In parallel size-fractionated water samples the attached and free living microbial community was compared to the roller tank incubations and the drifter trap samples. All results indicate that the bacterial community in TEP structures remains rather constant, but shows a strong temperature response during the sinking process. In contrast, the bacterial community on the aggregate itself is not constant and probably more influenced by the organic material than by external factors.

MMIV009

Identification of bacterial genes required for diatom-bacteria interactions during marine aggregate formation

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Marine aggregate formation is an important mechanism that mediates sinking of organic carbon. Diatom-bacteria interactions play an important role during this process by inducing secretion of different polymers, which increase the size and stickyness of marine aggregates. A bilateral model system consisting of the diatom Thalassosira weissflogii and the Gammaproteobacterium, Marinobacter adhaerens HP15, is used to study diatom-bacteria interactions. HP15 specifically attaches to T. weissflogii cells, induces transparent exopolymeric particle formation, and leads to diatom aggregation. In the current work we identified bacterial genes and proteins induced during this interaction with T. weissflogii. An In vivo expression technology screening was performed. A vector had to be constructed, which carried the promoterless lacZ gene downstream of a promoterless pyrB gene encoding for an essential growth factor. Additionally, a pyrB-deficient mutant of M. adhaerens was generated, which was unable to grow in the absence of uracil and in presence of the diatom. A genomic DNA library was cloned into the vector and transformed into the mutant. The transformants were co-cultured with diatoms selecting for cells expressing pyrB. The DNA fragments, which did not lead to lacZ expression in vitro, were sequenced to identify genes under their control. In a second approach, protein profiles of M. adhaerens HP15 co-cultured with diatoms or grown solo were compared by two-dimensional gel electrophoresis coupled with MALDI-TOF analysis. The identified bacterial genes and proteins will further be characterized by mutagenesis. Results of this study will contribute to a better understanding of the molecular mechanisms underlying diatom-bacteria interactions.

MMIV010

Bacterial community associated with Ctenophores at Helgoland Roads, German Bight

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Question: Large increases in gelatinous zooplankton populations have occurred in many coastal and estuarine ecosystems worldwide because of eutrophication, climate change, and anthropogenic activities which have greatly altered the natural marine environment. Jellyfish release nutrients and dissolved organic matter which may stimulate bacterioplankton growth. However information about the community structure of the bacteria associated with ctenophores is rare.
Methods: Four ctenophores species *Mnemiopsis leidyi*, *Beroe* sp., *Bolinopsis infundibulum* and *Pleurobrachia pileus* were sampled at Helgoland Roads in the Germany Bight, North Sea from Oct.2009 to Oct. 2010. Each individual was washed 5 times with sterile seawater to remove transient and loosely associated microorganisms from the surfaces of ctenophores. Automated Ribosomal Intergenic Spacer Analysis (ARISA) fingerprinting and 16S ribosomal amplicon pyrosequencing were performed to investigate the structure and diversity of the bacterial communities associated with ctenophores.
Results: We revealed significant differences between associated-bacterial communities among different ctenophores species based on ARISA fingerprinting (P<0.001). Based on 16S ribosomal amplicon pyrosequencing, Proteobacteria (87%-98%) were the majority of the

predominant bacteria in ctenophores. Within the Proteobacteria, *M. leidyi* and *P. pileus* mainly harbored Gammaproteobacteria, *Marinomonas* made up the majority of the predominant Gammaproteobacteria in *M. leidyi*. Conversely, in *P. pileus* the Gammaproteobacteria were mainly composed of *Pseudoalteromonas* and *Psychrobacter*. The bacterial community of *B. infundibulum* comprised of 46.5% Alphaproteobacteria and 52.71% Gammaproteobacteria which in specific were *Thalassospira* and *Marinomonas*. *Beroe* sp. was mainly dominated by Alphaproteobacteria, particularly by *Thalassospira*.

Conclusion: Bacterial communities associated with ctenophores displayed a low diverse and highly species-specific character. The genus *Marinomonas*, *Pseudoalteromonas*, *Psychrobacter* and *Thalassospira* are the predominant groups.

- Bacterial communities associated with ctenophores were consistently divergent from those presented in the surrounding sea water which has been reported before.

MMIV011

Plastic pollution and marine microbes: Exploring the plastic colonising community in the ocean

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Plastic particles have been accumulating in the marine environment for over 100 years. But the alarming scale of this pollution has only recently been recognized, e.g. through the discovery of the Pacific and Atlantic Garbage Patches. Plastic particles can enter the marine food web and affect organisms at all levels, including microorganisms. Microorganisms are known to adapt quickly to emerging niches and may use plastic as a habitat. Little is known about the role of microbes in the fate of marine plastic and associated co-pollutants, and the consequences of plastic pollution on marine microbial communities. Our study aims to characterise the structure, diversity and function of microbial communities colonising plastic. We attached PET bottles for a six-week period onto buoys in theNorth Sea. We also performed Manta trawl sampling in the North andBalticSeas, to collect plastic pieces directly from the environment. Plastic pieces were then checked for microbial colonisation using fluorescence microscopy and scanning electron microscopy (SEM). Both 16S and 18S rRNA genes were amplified from DNA from plastic fragments. To investigate spatial and seasonal differences in the structure of the plastic-colonising community, DGGE fingerprinting was carried out. rRNA genes from DGGE bands were sequenced to identify microbial plastic colonisers. We discovered a specific microbial community colonising plastic, differing from those colonising glass or present in background water. Spatial and seasonal variation was observed, but certain microbial groups, such as Flavobacteria and Bacillariophyceae, were abundant on all plastic samples. Most microorganisms identified were related to those that typically form biofilms. To get more in-depth information on the structure and function of the plastic-colonising communities, high-throughput sequencing will be performed.

MMIP001

A marine coculture of archaeal ammonia oxidizers and nitrite oxidizers enriched from the North Sea

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Although nitrification was previously assumed to be mediated by bacteria, archaeal ammonium oxidizers (AOA) have been postulated as key players in marine ecosystems. Elucidation of AOA physiology is hampered due to technical difficulties in growing these microorganisms in sufficiently high quantities.

The aim of our study was to enrich AOA together with nitrite-oxidizing bacteria (NOB) from North Sea water. First a batch flask enrichment culture was established by incubation of tenfold concentrated and 0.45 μm filtered North Sea water with 200 μM ammonium (NH_4Cl). Ammonium was replenished upon depletion and whenever the nitrite concentration exceeded 200 μM half of the culture volume was replenished to minimize nitrite toxicity. The average ammonium consumption rate was 1 μmole per day under these conditions. Counter intuitively, this rate increased tenfold after switching to a static incubation.

Batch culture material was subsequently used to inoculate a 1L bioreactor amended with 30 μM NH_4Cl. This reactor was operated without agitation at room temperature under reduced oxygen conditions. Initially a batch operation was adopted and ammonium was converted in a 1:1 ratio to nitrite. After 4 times of replenishing NH_4Cl in batch operation, nitrite was no longer detectable indicating that both AOA as well as NOB were active. Hereafter, the reactor volume was increased to 2L by influx of medium containing 150 μM NH_4Cl after which a continuous mode of operation adopted (D= 0.03). The ammonium concentration in the influent was increased in a stepwise manner to 2 mM NH_4Cl leading to an increase of ammonium consumption rate from 1 μmole (day 5) to 100 μmoles per day (day 455). Results from amplifications using primers targeting either bacterial or archaeal *amo*A genes indicated that only archaeal ammonia oxidizers were present. Metagenomic sequencing revealed the community consists mainly of *Nitrosoarchaeum limnia* AOA with a new species of *Nitrospina* as the nitrite-oxidizing partner. This bioreactor is expected to yield sufficient North Sea AOA biomass to allow for more in-depth physiological characterization (e.g. pH, T optima, affinity constants for oxygen and ammonium) and allow for comparison of this AOA species to other AOA such as *Nitrosopumilus maritimus* strain SCM1.

MMIP002

Can anaerobic oxidation of ammonium be coupled to sulfate reduction in marine enrichment cultures?

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Anaerobic oxidation of ammonium is a major process responsible for nitrogen loss from the Ocean. So far, ammonium oxidation in anoxic marine environments has been only shown to be coupled to reduction of nitrite. However, current geochemical evidence suggests that ammonium is lost from anoxic sediments where no oxygen, nitrate or nitrite is present. Interestingly, ammonium disappears in the same zone in which sulfate is reduced. It has therefore been proposed that lithotrophic ammonium oxidation coupled to sulfate reduction can also be responsible for ammonium removal. Geochemical profiles suggest that the sulfate-coupled ammonium oxidation could attribute for up to 3% of the total sulfate reduction in the ammonium-sulfate transition zone (Schrum *et al*., 2009). These zones are dominated by Deltaproteobacteria and methanotrophic archaea capable of sulfate-coupled anaerobic oxidation of methane (AOM), which is the main process for sulfate reduction in this depth.

Using a combination of stable isotope labelling, analytical and single-cell techniques we have investigated whether our AOM enrichment cultures dominated by ANME-2 and *Desulfosarcina*-related bacteria can also oxidize ammonium under anaerobic conditions.

Our preliminary results suggest that small, but detectable, amounts of ammonium-derived, intermediate oxidation state nitrogen compounds are transiently formed in the AOM enrichment culture. This reaction only occurs when methane is present in the incubation.

Sulfate-reducing ammonium oxidation in marine sediments might therefore represent a novel sink for fixed nitrogen and a new connection between the biogeochemical sulfur and nitrogen cycles.

Schrum H.N. *et al*. (2009) Sulfate-reducing ammonium oxidation: A thermodynamically feasible metabolic pathway in subseafloor sediment. *Geology* **37**(10): 939-942.

MMIP003

Microbial community diversity in sediments from the Skagerrak and Bothnian Bay

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The biogeochemical cycling of iron is a key process in terrestrial and aquatic systems including marine environments. However, limited information exists about the diversity and metabolic pathways of microorganisms linked to the iron cycle in ocean sediments. Past studies have shown the Skagerrak (SK) to harbor microorganisms capable of high rates of iron reduction (1). Geochemical studies have also shown the Bothnian Bay (BB) to be rich in Mn and Fe nodules (2). In this study, DNA/RNA were extracted from various depths from sediments collected in SK and BB. Porewater profiles of relevant electron acceptors showed that sulfate was ~28 mM in SK sediments and ~ 2 mM in the BB sediments at all depths. In addition, H_2S levels were below detection in both sediments, and Fe^{2+} and Mn^{2+} levels reached ~ 140-150 μM in SK sediments between 6-7 cm depth and ~300 μM

within the first 2 cm in BB sediments. V1-V3 regions of the 16S rRNA gene were determined using 454 titanium pyrosequencing and sequences analyzed using QIIME (3). Both sediments were enriched primarily with Proteobacteria. The most studied iron reducing bacteria belong to the δ- and γ-Proteobacterial classes including *Shewanellaceae* and *Geobacteraceae* families. BB sediments from 3-4 and 7-8 cm depths show Proteobacteria composition to be evenly distributed among the α-,β-,γ-, and δ-Proteobacteria. Within the δ-Proteobacteria, most abundant were members of NBJ-1, Myxcoccales, and Syntrophobacterales. Within the γ-Proteobacteria most abundant were members of the Chromatiales and Oceanospirillales. A recent study showed that Oceanospirillales are involved in acetate dependent manganese reduction (4). In contrast SK sediments from 6-8 cm depth show the Proteobacteria is dominated by α, γ and δ-Proteobacteria. Similar to the BB, SK sediments showed the presence of Chromatilaes and Oceanospirllales. Rarefaction analyses indicate the overall diversity within each sample was captured sufficiently with several thousand titanium sequences and microbial diversity is significantly different between SK and BB samples. Together these results suggest both SK and BB sediments may harbor unique microorganisms capable of iron reduction metabolism.

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MMIP004

Diversity, ecology, and physiology of the *Roseobacter* clade and other marine microbes in the North Sea

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Marine bacterioplankton communities play major roles in many ecological important pathways such as the global carbon cycle. One widely distributed and abundant planktonic group is the *Roseobacter* lineage. The use of a multitude of different organic compounds, the production of secondary metabolites, and other metabolic pathways contribute to the success of this group in a variety of different marine environments.
Our studies were focused on assessing and exploiting the diversity, ecology, and physiology of members of the *Roseobacter* clade and other marine microorganisms. For this purpose, samples derived from the North Sea were investigated employing metagenomic and metatranscriptomic approaches.
Total DNA and RNA were extracted from each sample and processed in different ways. To assess the microbial diversity, DNA and RNA were subjected to 16S rRNA gene PCRs and 16S rRNA RT-PCRs, respectively. Obtained PCR products were sequenced and further analyzed. To reveal the genomic potential and actively transcribed genes, isolated DNA and cDNA, derived from mRNA-enriched environmental RNA, were sequenced employing next-generation sequencing.
We identified members of the *Roseobacter* lineage as one very abundant and active group of the investigated marine bacterioplankton communities. Other prominent marine groups found in many investigated samples were the SAR116 group, SAR92 group, and members of the *Flavobacteria*. We recorded significant changes in active community structures with respect to environmental conditions, e.g., the presence of an algal bloom. Moreover, we were able to reconstruct functional traits by mapping mRNA-derived sequences onto available genomes of different cultured marine bacteria, such as Candidatus *Planktomarina temperata*, the only sequenced representative of the *Roseobacter* RCA subclade.

MMIP005

Cultivation of terrestrial DOC degrading bacteria from the Baltic Sea

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A large amount of terrestrial dissolved organic carbon (tDOC) is accumulated in permafrost soils of the northern hemisphere. Concomitant with climate change, tDOC is mobilized by melting of Permafrost and enters marine habitats. Only little is known of the mechanisms of tDOC degradation in marine habitats. Because of its unique salinity gradient that ranges from nearly limnic to marine conditions and since it has been shown that the bacterial community changes along this salinity gradient, the Baltic Sea represents a suitable model system for tDOC degradation under different environmental conditions. In order to investigate the basis of tDOC degradation, key bacterial species were isolated. Long-term incubation experiments were established with water from the Kalix River (next to Överkalix, North Sweden) as tDOC source. To study the effect of salinity on tDOC degradation, Baltic Sea water with three different salinities (32 g/l, 7 g/l and 2 g/l) were used as inoculum. To isolate the relevant tDOC degrading bacteria, samples were taken during different stages of tDOC degradation and processed with the MultiDrop technique for high throughput cultivation. The changes of cultivability of potential tDOC degrading bacteria was quantified with the most probable number method. First results show a higher cultivability of the incubation experiment with a salinity lower than 7 g/l , in which cultivability reached up to 25% of total bacterial cells. The community composition of cultivable bacteria was assessed by DGGE-fingerprinting of 16S rRNA genes. Sequence analysis and physiological characterization yielded information on the potential for tDOC degradation of the isolates.

MMIP006

High-throughput isolation of *Phaeobacter*-related members of the *Roseobacter* clade from diverse marine samples

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Bacteria of the *Roseobacter* clade represent one of the most abundant groups of marine bacteria. They may account for 50% of all bacterial phylotypes present and are characterized by a large phylogenetic and physiologic diversity. So far, the evolutionary mechanisms underlying the divergence of the *Roseobacter* clade are unknown. Members of the genus *Phaeobacter* have several particular physiologic features including the production of AHLs, algaecides and the antibiotic tropodithiethic acid. In addition, they can switch from free-living to surface-associated mode of growth and contain numerous plasmids. This genus therefore represents a suitable model for the study of recent evolutionary events within the *Roseobacter* clade. In order to elucidate these events by population genetics approaches, a broad range of strains from diverse marine habitats is needed. However, only few members of the genus are sufficiently characterized. Therefore, we performed liquid and biofilm-specific high-throughput isolation strategies in defined oligotrophic media with samples from a range of 20 different marine habitats. The success of enrichment was screened via a specific PCR targeting all *Phaeobacter* and closely related strains. Our isolation strategies yielded about 500 (4% of all grown enrichments) strains of *Phaeobacter* and relatives. Only few target organisms could be enriched from water and anorganic surfaces, whereas a much larger fraction of positive cultures (16%) was enriched from zooplankton. Different phylotypes were enriched from different habitats. For instance, many *Pelagicola litoralis* strains were enriched from zooplankton of the North Sea and *Leisingera nanhaiensis* strains were obtained from mussels. *Phaeobacter arcticus* but no *P. litoralis* strains could be successfully enriched from zooplankton of the Baltic Sea, indicating a possible habitat specificity of the different lineages.

MMIP007

Occurrence of Roseobacter organisms in tropical sponges

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Sponges are hosts of diverse bacteria including unique but also more widespread phylogenetic lineages. The bacterial biomass can constitute up to 40 % of the total sponge biomass. Bacteria of the *Roseobacter* clade are widespread in marine ecosystems and habitats and can be highly abundant in some of them. Several organisms of this clade are capable of producing secondary metabolites and often live associated with various marine eukaryotic organisms. Even though roseobacters have previously been found in sponges, the diversity and role of these bacteria in their hosts is not well understood. Therefore, we investigated sponges from tropical seas for the occurrence of roseobacters, selected five different sponges for isolating roseobacters and studied their potential to produce secondary metabolites inhibiting growth of various bacteria.

MMIP008

Spatial distribution and diversity of the *Roseobacter* clade in temperate and permanently cold marine sediments

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The *Roseobacter* clade is regarded to be a major bacterial lineage in temperate and cold marine environments. *Roseobacter* affiliated bacteria represent a numerically significant part not only of pelagic, but also of benthic microbial communities. To understand their biogeography, diversity and specific metabolic adaptations, sediment and water samples were recovered during two cruises to the eastern North Sea and the Antarctic Peninsula.
North Sea samples were analyzed by *Roseobacter*-specific DGGE to identify regional differences and a putative overlap between free water, particles and the sediment surface. The cluster analysis of DGGE patterns revealed clear differences between free living and attached roseobacters with subpopulations found along the German/Danish coast and the Norwegian channel. Interestingly, particle-bound *Roseobacter* communities from young, surface-near layers clustered separately, while the community composition of old, matured particles showed high similarities to that of the sediment surface. The results of the DGGE analysis will further be confirmed by 454 high-throughput sequencing.
Apart from these cultivation-independent analyses, MPN enrichments in media amended with either DMS, DMS+lactate, DMSP and DMSO were performed to quantify the amount of roseobacters contributing to the sulfur cycle. All media were shown to be appropriate for growing members of the *Roseobacter* clade. Positive enrichments were selected for further isolation procedures.
For the Antarctic samples, total cell counts were determined by fluorescence microscopy. The percentage of *Roseobacter*-affiliated bacteria was quantified by CARD-FISH and verified by qPCR. A clear trend of decreasing cell numbers was observed from shallow to deep-water stations with an average percentage of roseobacters of up to 2%. In contrast to the MPN enrichments from the North Sea, the Antarctic samples showed high microbial growth predominantly in DMSP-amended dilution cultures.

MMIP009

First identification and characterisation of Rubber Oxygenase RoxA orthologues in Gram-negative bacteria

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Marine microorganisms recently came into focus of research activities. Most rubber degraders belong to the actinomycetes (e. g. *Gordonia, Streptomyces*) and rubber-degrading ability in these species correlates with the presence of the latex-clearing protein (Lcp) [1]. *Xanthomonas* sp. 35Y is the so far only known Gram-negative bacterium that is able to utilize rubber. It degrades poly(*cis-1,4*-isoprene) by secretion of rubber oxygenase RoxA, a novel type of dihaem dioxygenase that is not related to Lcp. [2, 3]. Here, we provide evidence that Gram-negative bacteria, 3 marine and one soil bacterium, possess RoxA orthologues and are likely to represent novel rubber degraders. A blast search revealed hypothetical proteins in the translated genomes of *Corallococcus coralloides*, *Myxococcus fulvus*, *Haliangium ochraceum* and *Chondromyces apiculatus* that were highly similar to RoxA. All species are myxobacteria and, except *C. apiculatus,* are living in marine environment. The identified sequences were 63-66 % identical to RoxA of *Xanthomonas* sp. and are characterized by two haem binding motifs for covalent attachment of haem and by other features known from RoxA. *H. ochraceum* was chosen for further analysis and turned out to produce clearing zones on solid latex agar. Recombinant $RoxA_{Hoc}$ was expressed in a Δ*roxA* background of *Xanthomonas* sp. First results of the secreted $RoxA_{Hoc}$ protein revealed the same characteristic features in the UVvis spectrum as had been previously described for $RoxA_{Xsp}$ [2, 3]. Notably, we confirmed polyisoprene-cleaving activity for $RoxA_{Hoc}$ by identification of 12-oxo-4,8-dimethyltrideca-4,8-diene-1-al (ODTD) as degradation product by HPLC.
We conclude that $RoxA_{Hoc}$ represents a RoxA orthologue. Based on the high amino acid similarity we postulate that the identified hypothetical proteins in *C. coralloides, M. fulvus, and C. apiculatus* also represent RoxA orthologues.

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MMIP010

Effects of ecological engineered oxygenation on the microbial community structure in an anoxic fjord in western Sweden

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In recent years extensive oxygen minimum zones (OMZ) have become increasingly common in marine ecosystems, often due to global warming and other anthropogenic influences. Considering the importance of oxygen for the distribution of organisms, this development has a high impact on the composition of microbial communities in pelagic ecosystems. Besides studying OMZs, more effort has recently been put into counteracting their spreading.
In order to remedy the long-term hypoxic or anoxic bottom waters of the Baltic Sea, the Baltic deep-water OXygenation (BOX) project proposed to introduce oxygen by using wind driven pumps to generate artificial deep-water ventilation. To test if the artificial oxygenation is feasible, a pilot study was initiated in 2009 as a part of the BOX project. The Byfjorden was chosen for this study because it is a stratified system with a lower water column and benthic zone that has been anoxic for a long time. Also, freshwater from a river supports a brackish, well-oxygenated surface region with lower salinity than the deeper layers, strengthening the stratification. With all these features, the Byfjorden mimics the Baltic Sea on a smaller scale and represents and ideal model system.
We used an electric pump to mix surface water into the deeper layers of the Byfjorden and could by this trigger several inflows of oxygen rich water into former anoxic areas. This increased oxygen levels in the lower water column and the benthic zone up to 110 μmol/l.
In this study we monitored changes in microbial community structure in response to the oxygenation project in the Byfjorden using a molecular microbial community fingerprinting method. We analyzed water column samples from before, during and after the oxygenation as well as from two nearby control fjords. Our results show a strong response of the microbial community to the oxygenation. Initially dominant indicator species for OMZs e.g. members of the SUP05 clade declined in abundance during the oxygenation event and vanished entirely after the oxygenation was accomplished. In contrast, aerobic species like e.g. SAR11 were after the oxygenation not only detected in surface waters but also deep into the water column. Here, we present the results of our study in the context of biogeochemical data to show the impact of the oxygenation on the microbial community.

MMIP011

Bacterial turnover of iodomethane - a climate-relevant trace gas - in Baltic and North Sea surface waters

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Iodomethane is a volatile halogenated organic compound and thus a strong source of highly reactive halogen oxide radicals in the atmosphere, which catalyse climate relevant processes like ozone-depletion. However, oceanic emission budgets of iodomethane are still poorly understood, partly due to large uncertainties about the equilibrium of biological production and decomposition processes. Iodomethane is known to be produced by several algae, e.g. diatoms and cyanobacteria. Additionally, abiotic processes such as hydrolysis or photochemical cleavage act as sinks and/or sources of iodomethane. Bacterial degradation of iodomethane has been considered to be another important factor, but studies are very limited.

Our aim of the present study was to determine the bacterial uptake of iodomethane using ^{14}C-labelled substrates and to identify bacterial organisms to be stimulated in iodomethane enrichment experiments. To further link the uptake of iodomethane with the identity of involved species, we applied RNA-based stable isotope probing experiments using ^{13}C-labelled iodomethane. Samples were taken in the central Baltic Sea (summer 2011) as well as in the coastal Baltic and North Sea (spring 2011).
The uptake rates of iodomethane in the Baltic Sea surface water ranged between 50 - 600 pmol h^{-1} L^{-1}, which was unexpectedly high regarding the *in situ* concentrations of iodomethane (5-30 pmol L^{-1}). We found that only few bacterial taxa were stimulated upon the addition of iodomethane. The dominant stimulated organism in the coastal Baltic Sea belonged to the *Methylophilaceae*, from which members were previously found to degrade halocarbons. In the coastal North Sea, the dominant stimulated organism was related to the *Oleispira*, which are known for their hydrocarbonoclastic physiology. However, subsequent stable isotope probing experiments could not prove the incorporation of ^{13}C-iodomethane in the RNA of these two organisms.
Although the incorporation of iodomethane by the stimulated organisms could not be detected so far, our results show that bacteria generally take up and probably degrade iodomethane in coastal waters. Future studies will focus on their identity to further elucidate the impact of bacterial degradation of halocarbons in aquatic habitats.

MMIP012
Understanding the relationship between Roseobacters and Dinoflagellates

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Roseobacters are heterotrophic alphaproteobacteria widely distributed in the marine environment. They play a very important role in global carbon and sulfur cycles [1] and are often found in association with dinoflagellates which may form harmful algal blooms. Some dinoflagellates require vitamin B_{12} for their growth, which can be produced by many Roseobacters. Dinoflagellates in return provide Roseobacters with carbon and energy sources. This relationship is still not well understood.
Here we studied the interactions between Roseobacters and Dinoflagellates using *Dinoroseobacter shibae* [2] and *Prorocentrum minimum* as model organisms. We carried out cocultivation experiments of these two organisms under controlled conditions and monitored the growth of algae and free-living bacteria using flow cytometry. Very distinct population dynamics of algae were observed in the absence and presence of *D. shibae* in vitamin B_{12}-limited and replete medium. The results indicate both stimulating and inhibitory effects of *D. shibae* on *P. minimum* depending on the growth stage of algae. To identify genes relevant for their relationship, we also performed transcriptome studies of the coculture during the light-dark cycles. RNA sequencing data of *D. shibae* showed that a phasin encoding gene comprised up approximately 10% of the transcripts. Phasins are major proteins present on the granules of polyhydroxyalkanoate (PHA) and are proposed to be involved in the regulation of PHA synthesis, degradation and granule size control. Comparison of gene expression in the light and in the dark showed upregulation of a transporter for dimethylsulfoniopropionate (DMSP) in the light. DMSP is synthesized by algae and functions as osmoprotectant or antistress molecule which can be used as sulphur and carbon source by bacteria.
In conclusion, phasin and DMSP transporter may play an important role in the survival of *D. shibae* and in the interaction with *P. minimum* in coculture.

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MMIP013
A hydrogen oxidizing enrichment culture as an analog to hydrothermal vent environments: understanding their function

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Primary biomass production at deep sea hydrothermal vents is largely driven by the microbial oxidation of inorganic electron donors (e.g. hydrogen) ascending with highly reduced and extreme hot hydrothermal fluids from inner earth. Due to mixing processes of these fluids with the ambient seawater steep physical and chemical gradients arise. Also, a multitude of habitats characterized by different conditions (e.g. temperature, oxygen and substrate availability) is provided, which can be colonized by phylogenetically and physiologically diverse microorganisms. The key enzymes for H_2 metabolism are hydrogenases (H_2ases), enzymes catalyzing the interconversion of molecular hydrogen to protons and electrons ($H_2 \leftrightarrow 2H^+ + 2e^-$).
Here we describe an enrichment culture for microaerophilic autotrophic hydrogen oxidizers. To set up the enrichment culture a sample of diffuse fluids from Sisters Peak (5°S on the Mid-Atlantic Ridge) was taken and incubated in artificial seawater supplemented with vitamins, trace elements and bicarbonate under an atmosphere of H_2:CO_2:O_2 (79:20:1) at 28°C.
To determine the culture community, 16S rRNA genes were analyzed. This enrichment culture consists of four different bacteria, namely *Thiomicrospira crunogena*, *Alteromonas macleodii*, *Pseudomonas pachastrellae* (*Gammaproteobacteria*) and *Thalassospira sp.* (*Alphaproteobacteria*). Archaeal 16S rRNA genes could not be amplified. Interestingly, from literature we know H_2ase genes from some of these species, which we also amplified, but growth with H_2 has not been described. Investigating the mixed culture, clear uptake H_2ase activity of membrane associated proteins was shown (0.25±0.07 μmol H_2*min^{-1}*mg^{-1}), soluble proteins exhibited only very low activity. Also, H_2 consumption during growth of the culture was determined; both demonstrating the expression of active H_2ases by this enrichment. Unfortunately, assigning these H_2ases to one of the species has not been successful yet. To identify the H_2ase expressing species, we investigate the isolated species with respect to H_2 utilization and sequence active H_2ases expressed by the mixed culture. Furthermore, NanoSIMS experiments in combination with *in situ* hybridizations are planned to find out more about H_2 and also CO_2 fluxes and to link the physiology with the phylogeny.

MMIP014
Functional analysis of sulfide: quinone oxidoreductase homolog in *Sulfurimonas denitrificans*

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Hydrothermal fluids are rich in reduced inorganic substrates (e.g. sulfide) and support a large population of chemolithotrophic bacteria. Some of these bacteria can oxidize reduced sulfur compounds, particularly hydrogen sulfide, a chemical highly toxic to most known organisms. The energy gained from the biological oxidation of sulfide can then be used to fuel cellular processes. An enzyme that can catalyze the oxidation of sulfide is Sulfide:quinone oxidoreductase (SQR). Our previous metagenomic studies have revealed a high diversity of genes encoding the SQR enzyme in distinct hydrothermal vents. Most of these *sqr*-genes are highly similar to the *sqr*-gene from the ε*-proteobacterium Sulfurimonas denitrificans*. Here we show that the *sqr*-gene in *S. denitrificans* is transcribed, expressed and the SQR enzyme is functional. SQR assays were performed with the membrane proteins, integral membrane proteins and peripherally bound inner/outer membrane proteins isolated from *S. denitrificans*. The highest SQR activity among the tested protein fractions was from the integral membrane protein. This suggests that the SQR of *S. denitrificans* is localized in the periplasmic space. The SQR activity in the integral membrane protein from *S. denitrificans* is much higher than the activity of other tested SQRs. As a next step we envisage to investigate whether the *sqr*-genes recovered from the hydrothermal vent sites are functional. For this purpose we will heterologously express the environmental *sqr*-genes and test their activity.

MMIP015
Diversity of Rhodopirellula spp. in costal sediments

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Over 70 strains of *Rhodopirellula spp*. were isolated from European Seas (1) and characterized with a multilocus sequence analysis combined with DNA-DNA hybridization experiments (2). These studies demonstrated the presence of several *Rhodopirellula* species with *R. baltica* (operational taxonomic unit A (OTU A)) occurring in the Eastern North Sea and the Baltic Sea, OTU B in the Mediterranean Sea and the British Channel and OTU I in Iceland and Scotland. The MLSA characterization of *Rhodopirellula* isolates has revealed a broad genetic diversity in European seas but also clearly demonstrated that conventional 16S rRNA comparison alone was not sufficient to resolve the genetic separation of *Rhodopirellula* isolates from different habitats. Therefore, novel genetic markers are

required to resolve the genetic diversity of *Rhodopirellula* communities from environmental samples. To gain access to more data, the genomes of eight *Rhodopirellula* isolates were sequenced. The eight strains where used for the identification of suitable marker genes. For the diversity study in sandy coarse sediments of Sylt Island, Germany, *carB* primer was used for targeting the amplicon of this functional gene of *Rhodopirellula* and construction of clone libraries. In addition, 16S rRNA clone library with Planctomycete specific primer was constructed. Analysis of the *carB* clone libraries show high local diversity of Rhodopirellula spp. in Sylt sediments which could not be shown with the analysis of 16S rRNA gene clone library due to the low taxonomic resolution of this gene above the species level.

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MMIP016

Elucidating particle break-down and biochemical potential of the diatom-associated bacterium *Marino-bacter adhaerens* using proteomics

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This proteomics-based project aimed at the elucidation of extracellular proteins of *Marinobacter adhaerens* HP15 assumed to act on macromolecular structures and thus being of ecological relevance during marine aggregate formation and break-down.
The heterotrophic bacterium *Marinobacter adhaerens* HP15 was isolated from marine particles of the German Wadden Sea, found to actively attach to cells of the diatom *Thalassiosira weissflogii*, and to induce marine aggregate formation. Based on these findings, a genetically accessible model system for bacterium-diatom interactions consisting of HP15 and *T. weissflogii* was established. This systems allows the functional study of individual bacterial genes by genome analysis and mutagenesis. In the marine water column heterotrophic bacteria such as *M. adhaerens* may contribute to the consumption and re-mineralization of marine particulate and dissolved organic matter, which are important processes in marine nutrient cycling. Uptake of particles into a bacterial cell is limited by particle size (< 600 Da). Consequently macro-molecular compounds need to be first broken down to smaller sizes in the cell's exterior. It was assumed that hydrolytic proteins are present in the extracellular space, in or attached to membranes, or in the periplasmic space of bacterial cells, and that expression of those proteins might be substrate-dependent. To identify such proteins, the bacterium was incubated *in vitro* with a set of marine macromolecular carbon sources. When HP15 cells multiplied using these carbon sources, proteins derived from the cellular compartments were isolated and subjected to matrix assisted laser desorption/ionization - time of flight mass spectrometry. Initial results of this analysis will be discussed in context of marine biogeochemical processes.

MMIP017

Genomic and physiological analysis of *Pseudovibrio* sp. FO-BEG1 reveals a versatile metabolism, production of secondary metabolites, and potential mechanisms for interactions between bacteria and sponges

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The majority of strains belonging to the genus *Pseudovibrio* have been isolated from marine invertebrates like tunicates, corals and especially sponges, but the physiology of these bacteria is poorly understood. In this study, we analyze the genome of the *Pseudovibrio* strain FO-BEG1, which is a required symbiont of a cultivated *Beggiatoa* strain, and has been initially sampled from a black band diseased coral. The data show that the strain is a generalistic bacterium capable of importing and oxidizing a wide range of organic and inorganic compounds to meet its carbon, nitrogen, phosphorous and energy requirements under oxic and anoxic conditions. The physiological traits encoded in the genome of strain FO-BEG1 were also verified in laboratory experiments, confirming the genomic predictions. Besides the versatile metabolic abilities of this *Pseudovibrio* strain, our study reveals a number of open reading frames and gene clusters in the genomes that seem to be involved in symbiont-host interactions. *Pseudovibrio* sp. FO-BEG1 has the genomic potential to attach to host cells, might be capable of interacting with the eukaryotic cell machinery via type III and type VI secretion systems, produce secondary metabolites like the cytostatic metabolite colibactin and the antibiotic tropodithietic acid (TDA), and may supply the host with cofactors. In summary, our work is the first detailed analysis of the genomic and physiological abilities of a strain belonging to the *Pseudovibrio* genus. The results give us new insights into the astonishing, unexpected metabolic versatility of strain FO-BEG1. Furthermore, the genome of the investigated strain shows adaptations to an associated life-style, which expands our knowledge about possible mechanisms involved in the establishment and maintenance of associations of microbes and marine invertebrates.

MMIP018

Role of particle associated bacteria from the Baltic Sea in the degradation of terrestrial organic matter

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Permafrost soils contain around 60 % of global soil derived carbon. Current climate change has a strong influence on the arctic zones and is expected to result in a significantly enhanced mobilization of organic material which potentially drains into the sea. The impact of increasing terrestrial carbon on the global carbon budgets and shifts in community structures in marine habitats needs to be verified. Surprisingly, little is known on composition and activities of microbial communities involved in humic matter degradation. We hypothesize that particle associated microorganisms, in particular fungi, will benefit from and the increased input of terrestrial organic matter and thereby increase their ecological role in aquatic foods webs. Based on the fact that aggregates may contain large amounts of terrestrial organic matter, availability of microbial substrates can largely increase by exoenzymes produced by specific microorganisms. This leads to a decisive competitive edge against free living microorganisms. The Baltic Sea represents a suitable model system to study the degradation of terrestrial organic matter under different environmental conditions since it provides an extended salinity gradient from marine to almost limnic conditions and has long retention times varying between 3 to 30 years. To test for the role of terrestrial organic matter in food webs at different salinities, long-term incubation experiments were established with Baltic Sea water from 3 salinities (marine 32 PSU, brackish 7 PSU & limnic 2 PSU) as inoculum mixed with sterile Kalix River water (North Sweden) as a terrestrial organic matter source. Measured parameters of each salinity treatment were analyzed in triplicates. To improve the analysis of particle associated bacteria an additionally rolling tank experiment was performed. Microbial abundances were analyzed by Epifluorescence Microscopy, and Pyro-Sequencing was used to define aquatic microbial community compositions. The influence of particle associated bacteria and fungi on organic matter degradation was analyzed by comparing microbial data with organic matter finger prints. First results reveal specific changes in microbial community composition closely related to the different stages in degradation of the added terrestrial organic matter.

MMIP019

Syntrophic acetate oxidation between marine strains of sulphate reducers and methanogens: Testing the hypothesis

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Acetate is a key intermediate of organic matter degradation in anoxic environments and an important energy substrate for the microbial processes of sulfate reduction and methanogenesis. Unlike methanogenesis from hydrogen, methanogenesis from acetate tends to be thermodynamically favorable throughout sulfate-reducing sediment. Experiments with "non-competitive substrates" have suggested the possibility of methanogens getting involved in a syntrophic pathway of acetate oxidation. According to this hypothesis, acetate oxidation by methanogens is coupled to sulfate

reduction by interspecies hydrogen transfer (Phelps et al. 1985, Finke et al. 2007).
To test the hypothesis of syntrophic acetate oxidation of several methanogens and sulfate reducers, batch incubations of different combinations of acetotrophic and hydrogenotrophic strains were set up using 2-^{13}C-acetate as carbon source. The co-culture combinations included 1) *Methanosaeta concilii* DSM 2139 (aceticlastic methanogen) with *Desulfovibrio vulgaris* DSM 1744 (hydrogenotrophic sulfate reducer) and 2) *M. concilii* DSM 2139 with *Methanococcus maripaludis* S2 (hydrogenotrophic methanogen). We used High-Performance Liquid Chromatography and Gas Chromatography analyses to monitor the consumption of substrates and production of gases. IRMS will be used to determine the production of $^{13}CH_4$ and $^{13}CO_2$. Quantitative PCR (qPCR) will be performed to quantify the growth of all partners in the co-cultures.
The results obtained so far leave open the possibility of syntrophic acetate oxidation between combinations of sulfate reducers and methanogens.

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MMIP020

Micromonospora spp. strains from the deep sea as promising sources for new bioactive compounds

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Members of the *Actinomycetales* are widely distributed in terrestrial as well as aquatic ecosystems, where they are supposed to play an important role in the decomposition and recycling of biomaterials. They even have been recovered from the world's deepest ocean trench, the Mariana Trench. Evidence accumulates that they are active members of the microbial community in the marine sediment. A specific feature of the actinomycetes is their enormous contribution to the production of chemically diverse secondary metabolites and clinically relevant antibiotics.
The exploration of marine habitats with presumably new bacterial taxa is supposed to result in the discovery of unknown bioactive natural products. Therefore, we classified strains of actinomycetes isolated form the Eastern Mediterraneandeep-sea sediment by phylogenetic analysis and characterise their potential to produce bioactive compounds. Our results show that the deep sea strains are phylogenetic separated from their related type strains which might indicate specific marine clades. A considerable amount of the strains inhibited the growth of the Gram-positive bacteria *Bacillus subtilis* and/or *Staphylococcus lentus* and revealed to have the genetic potential to produce secondary metabolites by the nonribosomal polypeptide-synthase or the polyketide synthetase pathway. Furthermore, the production of novel bioactive compounds, like Levantilide A and B, demonstrate that the deep sea strains are promising producers of novel bioactive natural products [1].

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MMIP021

Linkages between decomposition of terrigenous dissolved organic matter (DOM) and microbial community structure: influences of chemical DOM characteristics

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The quality of dissolved organic matter (DOM) has been shown to influence bacterial community structure and subsequent the degradation of DOM. In this study we used a riverine DOM source to generate terrestrial DOM fractions of different quality which were mixed with a brackish bacterial community from the Baltic Sea. DOM was either added as particle-free river water (0.22 μm filtered) or modified by lyophilization and ultrafiltration. Dissolved organic carbon (DOC) consumption, DOM composition, microbial activities and shifts in the microbial community structure were compared to unamended Baltic Sea water and river water control experiments over a four week incubation period. The molecular composition of DOM, analyzed by ultrahigh-resolution mass spectrometry, indicated a clear difference of the DOM composition in the set-ups. During the course of the experiments we measured an average of 8% DOC loss. However, the molecular composition of DOM, which included several thousand molecular formulae, did not change significantly in any of the experiments. This may be due to degradation of colloidal or low-molecular weight DOM (<150 Da), that both escape the analytical window of the mass spectrometry. In contrast to DOM, bacterial numbers, activities and community compositions experienced drastic changes during the experiment. Whereas typical bacteria of the Baltic Sea were identified in the first days of the experiment, a gammaproteobacterial sequence previously found in hydrocarbon degrading studies became dominant after one week in all Baltic Sea treatments including the controls. The freshwater control experiment (where the added DOM originates from) experienced a shift towards a betaproteobacterial and flavobacterial dominated bacterial community that shows no similarity with the brackish bacterial community. This indicates that the addition of terrestrial DOM, probably due to the mainly refractory nature, has only minor effects on the change of the brackish bacterial community and that presumably only low-molecular weight DOM were utilized in the experiments.

MMIP022

Viral impact on microbiology and geochemistry of extremely nutrient-depleted sediments

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Large oceanic provinces such as the mid-ocean gyres are characterized by extremely low microbial activities within the water column and the underlying sediments. To understand the recycling of labile organic matter within subsurface sediments of the South Pacific Gyre (SPG), we have studied the impact of phages on this extremely nutrient-depleted habitat. We hypothesize that viral lysis might be one controlling factor for nutrient availability, microbial abundance and diversity.
In our cultivation-based approach, we have started to isolate subseafloor microorganisms from the SPG to identify prophages within their genomes. Samples that were recovered from different sites and sediment horizons were transferred into growth media onboard the drillship *JOIDES Resolution* to target either aerobic and anaerobic heterotrophs or autotrophs. Due to slow growth, we have now obtained the first isolates from a limited number of enrichments. Those are affiliated to *Nocardioides basaltis* (99% 16S rRNA sim.), *Halomonas aquamarina* (100%), *Erythrobacter vulgaris* (99%), *Pseudoalteromonas* sp. (99%), *Alteromonas* sp. (100%), and *Dietzia* sp. (100%). Their presence was additionally detected by molecular screening of the cultures by DGGE. Subsequently, we will start to induce the prophages within their genomes[1]. The morphologic and phylogenetic diversity of these phages will be analysed in detail. Concerning our culture collection, we will then identify if the percentage of infected cells that are under severe starvation is different to those isolated from high activity sites[2].
The diversity of viruses within the SPG sediments will be analysed by applying a novel approach using whole genome amplification. This technique allows to enhance the amount of viral DNA in sediments with an extremely low viral abundance. Subsequently, other molecular downstream applications such as RAPD-PCR will be used to analyse virus diversity patterns of the different sites.

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MMIP023

Habitat specific provocation of secondary metabolite production by marine bacteria - microbial communication and pharmaceutical use

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Sediments are an important habitat for the degradation of biopolymers and further nutrients in the marine food chain. Furthermore there are also extreme habitats in marine sediments, like hydrothermal vent fields, mud

volcanoes and cold seeps. These sediments do not have a terrestrial equivalent, so the examination of the microbiological diversity and the metabolic potential of the microorganisms will lead to new and promising secondary metabolites.
Most marine isolates are cultivated in standard growth media to obtain and examine secondary metabolites. However, genomic results indicate a larger potential for syntheses. The application of conditions that are designed based on environmental data is rarely studied. We focused on the provocation of metabolite production by applying habitat specific conditions for the cultivation experiments. The taxon specificity of the effect using different parameters was observed. Cultivation in "standard" media leads to interesting compounds as well. Hence, the application of a broad variety of media results in a broad range of metabolites. As an example, the *Micromonospora* sp. strain S20 from a deep sea sediment produces a new metabolite and derivatives thereof. These compounds showed an antibiotic effect against *Bacillus* sp. strains. Members of the genus *Bacillus* were dominant in the examined sediment samples. Applying habitat specific adaptation of the cultivation media revealed a strong increase in the production of derivatives. First results of the structure elucidation showed a large polyketide with a polyen partial structure.

MMIP024
Fermentative pathways for the energy generation in *Dinoroseobacter shibae*

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Fermentation processes are often essential for bacterial survival under conditions of oxygen limitation. It is an important strategy for energy generation without respiratory processes and enables bacteria to grow and survive under difficult environmental conditions. In this study *Dinoroseobacter shibae* was used as model organism. This marine bacterium exhibits an enormous metabolic versatility which serves as basis for ecological success. *D. shibae* belongs to the highly abundant and diverse Roseobacter clade, found in all marine habitats. With the genome annotation in 2009 genes for anaerobic pyruvate fermentation were found and indicated a mixed acid fermentation with a predicted conversion of pyruvate into lactate, acetate or to acetoin and butandiol. Surprisingly, *D. shibae* misses the gene *alsD* encoding the enzyme acetolactate carboxylase which converts acetolactate to acetoin. Therefore, we are interested whether *D. shibae* is able to use this part of the mixed acid fermentation. To unravel the supposed mixed acid fermentation pathways in *D. shibae* excreted fermentation products were identified and quantified via HPLC analyses. The supernatants of the wild type *D. shibae* DFL12^T as well as of different mutants showed pyruvate reduction to different products. Growth and survival assays with the wild type and knock-out mutants, lacking key enzymes of the pyruvate fermentation pathways were performed. Results confirmed the predicted pathway and demonstrated the contribution of the various branches to anaerobic survival. Metabolic flux analyses and results of microarray experiments will give further important insights to the fermentation metabolism. The data will be used to reconstruct the metabolic pathway of pyruvate fermentation in *D. shibae*.

MMIP025
Investigating direct pH effects on marine bacterial and fungal communities in microcosms

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The consequences of ocean acidification for heterotrophic marine bacteria remain under debate and almost nothing is known concerning marine fungi. Previous studies were carried out in large-scale mesocosms, where indirect effects mediated through complex food web interactions come into play. Here we present an alternative approach, using highly replicated microcosms (1-1.6 L). Focusing on bacteria and fungi, we wanted to investigate whether direct pH effects on community structure and abundances occur. Additionally we aimed to identify the groups reacting to pH reductions.
We incubated the natural microbial community from Helgoland Roads (North Sea) for four weeks at *in situ* seawater pH, pH 7.82 and pH 7.67. To reveal changes in community structure, we applied the fingerprint method Automated Ribosomal Intergenic Spacer Analysis (ARISA). Bacterial communities were furthermore analyzed by 16S ribosomal amplicon pyrosequencing. Abundances were determined by flow cytometry (bacteria) and colony forming unit counts (fungi). We repeated the experiments in different seasons and years and applied different dilution culture strategies to look at diversely assembled communities.
We predominantly found pH-dependent shifts in community structure at pH 7.82 for bacteria and at pH 7.67 for fungi. Bacterial abundance was not influenced by pH, while fungal numbers were on average 9 times higher at pH 7.82 and 34 times higher at pH 7.67. Different members of *Gammaproteobacteria*, *Flavobacteriaceae*, *Rhodobacteraceae* and *Campylobacteraceae* reacted to pH reductions. While *Rhodobacteraceae* were less characteristic for reduced pH, *Pseudoalteromonadaceae* and *Campylobacteraceae* profited from pH reductions. For most other bacterial groups, we did not observe consistent trends in the direction of the pH response.
Our findings demonstrate that already small reductions in pH have direct effects on bacterial and fungal communities. Although total bacterial abundance was not affected, both naturally abundant and rare bacterial groups reacted to pH reductions. The strong increase in fungal numbers at reduced pH suggests that fungi may reach higher importance in marine biogeochemical cycles and as infectious agents with ocean acidification. A 454 pyrosequencing approach is currently planned to resolve fungal identities.

MMIP026
Influence of increasing dissolved inorganic carbon concentrations and decreasing pH on chemolithoautrophic bacteria from oxic-sulfidic interfaces

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Increases in the dissolved inorganic carbon (DIC) concentration are expected to cause a decrease in the pH of ocean waters, a process known as ocean acidification. In this study, the potential effects of ocean acidification on chemolithoautotrophic prokaryotes in marine oxic-anoxic transition zones (redox zones) were investigated in the model chemoautotrophic denitrifying ε-proteobacterium "*Sulfurimonas gotlandica*" strain GD1. The bacterium uses reduced sulfur compounds, e.g., sulfide and thiosulfate, as electron donors and was previously shown to be responsible for nitrate removal and sulfide detoxification in redox zones of the Baltic Sea water column. Bacterial cell growth within a broad range of DIC concentrations and pH values was monitored and substrate utilization was determined. The results showed that the DIC saturation concentration for growth was already reached at 800 μM, which is well below in situ DIC levels. The pH optimum was between 6.6 and 8.0. Within a pH range of 6.6-7.1 there was no significant difference in substrate utilization; however, at lower pH values cell growth decreased sharply and cell-specific substrate consumption increased. These findings seem to rule out a direct effect of ocean acidification, with the predicted changes in pH and DIC, on chemolithoautotrophic bacteria such as "*S. gotlandica*" str. GD1. Instead, the effects of ocean acidification are likely to be indirect, but only over the long term.

MMIP027
Identification of the gammaproteobacterial sulfur-oxidizer SUP05 as a potential key organism in Baltic Sea pelagic redoxzones

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Gammaproteobacterial sulfur oxidizers (GSO), particularly SUP05-related sequences, have been found worldwide in numerous marine oxygen-deficient environments. However, knowledge is scarce regarding their abundance, distribution, and potential ecological role.
In the present study, we used 16S rRNA gene clone libraries and accordant phylogenetic analyses to identify the major gammaproteobacterial sulfur-oxidizers in Baltic Sea and Black Sea oxic anoxic transition zones (redoxzone). Specific oligonucleotide probes for CARD-FISH were designed to quantify their abundance throughout the oxygen gradient in different years and sampling stations. Additionally, RuBisCO form II (cbbM) gene transcript clone libraries were employed to detect potential active chemolithoautotrophic GSOs in the Baltic Sea.
Our results demonstrate the existence of two major phylogenetic subclusters embedded within the GSO cluster, closely related to sequences derived from various marine oxygen-minimum zones. The first affiliated with sequences

identified as potential autotrophs in the Black Sea (SUP05); and the second with potential autotrophs from the Baltic Sea. CARD-FISH analyses with newly designed specific oligonucleotides probe were used to quantify the *in situ* populations of these two groups. Only SUP05 occurred in higher numbers, reaching 15 -30 % of total prokaryotes (~ 10^5 cells mL^{-1}) in the Baltic Sea at depths where both oxygen and sulfide concentrations were minimal (below 5 μmol L^{-1}). The wider applicability of the oligonucleotide probes was confirmed with samples from the Black Sea redoxcline, in which SUP05 accounted for 10-13% of total prokaryotic abundance. *cbbM* transcripts potentially originating from SUP05 cells support previous evidence for the chemolithoautotrophic activity of this taxon.
Our results on the vertical distribution and high abundance of SUP05 suggest that this group plays an important role in marine redoxcline biogeochemistry, probably as anaerobic or aerobic sulfur-oxidizers.

MMIP028
Light sensitivity of methanotrophic bacteria

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Methane is the most abundant organic compound in the Earth's atmosphere and contributes to the greenhouse effect. Methane-oxidizing Bacteria (MOB) have the ability to use methane as a carbon source and energy source. Thus the emission of methane from natural sources is significantly reduced by the activity of MOB. Little is known about the influences of environmental factors on the microbial methane oxidation rates. Therefore the influence of UV-light on the MOX-rate was investigated in this work.
Methane oxidation rates were determined by tracer experiments with tritium labeled methane. Environmental samples as well as pure culture of Methylococcus luteus and Methylosinus trichosporium were incubated under UV-light and dark conditions.
First experiments indicate that the methane oxidation rate is inhibited by UV-light up to 40%. Additional other environmental factors like salinity, oxygen and temperature and their influence on the methane oxidation rate will be also investigated. The capacity to reduce methane concentrations in the environment of MOBs is mostly governed by methane itself, but other factors also may severely reduce this capacity.

MMIP029
Phenazine production by marine bacteria

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Phenazines show antibacterial, antifungal, antiviral and cytotoxic activities.[1,2] On the one hand, this class of metabolites presents a great potential for pharmaceutical applications. An example is clofazimine which is already established as an antileprosy agent.[3] On the other hand, phenazines are very important in the biocontrol of phytopathogenic fungi. *Pseudomonas chlororaphis* M342 for example is used for seed treatment against fungal diseases.[4] Due to the biotechnological applications of the phenazines, the aim of our study was the detection of phenazines producing bacteria from marine habitats. Two approaches were performed. The genetic approach includes PCR amplification of two *phz* gene fragments as markers for the ability to produce phenazines. The cultivation-based approach comprises cultivation and extraction of bacterial strains and subsequent chemical analysis with HPLC-MS. PCR amplification of *phz* gene fragments was accomplished for 56 bacterial strains comprising four control strains and 52 marine strains. 17 strains showed positive results using *phz*E primers[5] and only six strains exhibited *phz*F gene fragments.[6] These results clearly demonstrated that the application of the *phz*E primer system is a useful tool for the selection of phenazine producers from marine samples. In addition 28 of the 56 bacterial strains were cultivated, extracted and analysed by HPLC-MS analyses. Nine of these strains were identified as phenazine producers which correlates a rate of 53% of the *phz*E positive strains. Compared to the cultivation-based approach, it is already well known that the genome of bacterial strains contains more gene clusters which are forecasted to execute the biosynthesis of secondary metabolites.[7]
Thus, modification of the cultivation conditions might stimulate the production of phenazines in all *phz*E positive strains.

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MMIP030
Application of MALDI-TOF MS for environmental *Vibrio* surveillance programs

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Coastal areas of the North and Baltic Sea are threatened by potentially human-pathogenic vibrios like *V. cholerae*, *V. vulnificus* and *V. parahaemolyticus*. So far, outbreaks were generally reported to occur in regions with elevated water temperature. Due to global warming, an infiltration of Northern European temperate waters by perilous *Vibrio spp.* can be assumed. To assess this risk, environmental *Vibrio* strains have to be identified at least on the species level. In this context, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) seems to be a promising method because it provides extremely fast and accurate identification results. Hence, the main question of this study is whether MALDI-TOF MS can be an applicable analytical tool for *Vibrio* surveillance programs.
Mass spectra of one thousand *Vibrio* strains were generated with this proteomic fingerprinting method. Simultaneously all these isolates were characterized with approved *rpoB* sequence data analysis leading to a MALDI-TOF reference database filed with spectra entries, which are named after *rpoB* species identification results. A comparative examination is getting performed to check the validity of MALDI-TOF identification results and the distinctness for closely related *Vibrio* species. To evaluate this culture independent approach, about 2.100 MALDI-TOF samples were obtained from single agar plate colonies on a research cruise in 2012. These spectra will be matched with the new database to create a detailed report about the species composition of *Vibrio* populations in the North and Baltic Sea.
The comparative examination reveals that species-specific groups found by the *rpoB* sequence analysis are equal to MALDI-TOF cluster. In case of *V. alginolyticus* profiles, it was even possible to identify two different groups similar to *rpoB* cluster which were detected by previous investigations.
By MALDI-TOF MS, potentially pathogenic species like *V. vulnificus* can be clearly distinguished from closely related potentially non-pathogenic species like *V. navarrensis*. Hence, a fully created MALDI-TOF database facilitates the culture independent species detection of vibrios. And after a successfully evaluation of this database, MALDI-TOF could be implemented in future *Vibrio* surveillance programs.

MMIP031
Single-cell analysis of sulfur compounds in living bacteria by Raman spectroscopy

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Sulfur storage in bacteria was first documented by Sergei Winogradsky over a century ago, and yet our understanding of the chemical nature of stored sulfur is still fairly limited. A wide variety of bacteria capable of oxidizing reduced sulfur compounds store zero-valent sulfur intermediates either as intra- or extracellular globules. Conventional methods used to analyze these compounds rely on extraction; however, solvents as well as oxygen induce the chemical alteration biogenic sulfur compounds. Recently, the novel application of spectroscopic methods has indicated that the chemical speciation of stored sulfur might differ across groups of ecologically and physiologically distinct bacteria (Pasteris et al 2001; Prange *et al* 2002; He *et al* 2010). Based on these observations, it has been hypothesized that the chemical nature of sulfur globules may reflect different metabolic pathways utilized by the taxonomically diverse sulfur bacteria. To investigate this, we characterized and mapped the location of bacterial sulfur compounds in single cells of living bacteria using Raman spectroscopy. Preliminary results

suggest that the form of stored sulfur depends on the physiological state of the organism and might differ between groups of sulfide oxidizers.

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MMIP032

Bacteriophages in the deep marine subsurface

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Viruses are the most abundant biological entities on earth. In the oceans, they cause a short-cut in the carbon and nutrient cycling within the pelagic food web. The alteration of the pool of dissolved organic carbon and furthermore, the viral lysis of cells are shaping microbial community structures. A high viral turnover (0.15 to 8.2 d^{-1}) and the associated release of organic carbon were shown for the surface of deep-sea sediments, whereas their impact on the deep subsurface has been barely addressed. To better understand how viruses are influencing life and death of marine subsurface communities, we have now analyzed a globally distributed set of marine sediment samples for virus and prokaryotic abundance. The samples cover a broad range of sediment characteristics regarding TOC content, sedimentation rate, age and activity of indigenous microorganisms. By comparing abundances of viruses and prokaryotes in different subsurface environments and by elucidating their relationship, their potential impact on microbial communities will be discussed.
Considering the increasing recalcitrance of organic matter with depth, particularly in oligothophic and deeper sediments, the release of organic carbon after viral lysis of cells might be the most important viral impact on indigenous microorganisms.

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MMIP033

Cellular capabilities of "*Sulfurimonas gotlandica*" str. GD1 in redox gradients

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Oxic-anoxic interfaces of the pelagic central Baltic Sea are characterized by manifold redox-sensitive nutrient fluxes, which are relevant for biogeochemical cycles of the whole Baltic proper. Previous investigations revealed that their microbial communities are dominated by only a few, mainly chemoautotrophic, key microorganisms which catalyze important transformations within the nitrogen (N) and sulfur (S) cycle. In sulfidic waters below the oxic-anoxic interface epsilonproteobacteria of the *Sulfurimonas* subgroup GD17 are the major contributors to chemoautotrophic production. These bacteria are responsible for the oxidation of reduced sulfur compounds with nitrate by chemoautotrophic denitrification as the major nitrogen loss process in the pelagic central Baltic Sea. Only recently, the first representative of these habitats, "*S. gotlandica*" str. GD1, was successfully isolated from a Baltic Sea oxic-anoxic interface, serving as a model organism to answer the role of the GD17 cluster in N and S cycles of the central Baltic Sea. Aim of this study was to get more insight into cellular features of GD1, enabling it to move and survive within fluctuating pelagic redox systems. For this, motility behaviour as well as enrichment of GD1 cells in different S_2O_3 and NO_3 gradients was determined. In addition, potential cellular N and S enrichment or even storage were investigated by ^{15}N-NO_3 and ^{34}S-S_2SO_3 incubation experiments and coupled to NanoSIMS analyses.
After anoxic incubation in tube gradient systems, enrichment of GD1 cells were optically visible as white bands. Their extension increased with increasing substrate concentrations, potentially caused by overlapping gradients in the medium. Average speed of GD1 cells was 25μm s^{-1} with maximal speed well over 100μm s^{-1}. ^{34}S was intensely but ^{15}N only slightly enriched in GD1 cells. Thus, especially sulfur, but of unknown oxidation state yet, could potentially be stored in the cell and oxidized in nitrate-containing water masses.
We assume that these cellular capabilities of GD1 are indicative for their adaptation to micro turbulences in Baltic redoxzones, allowing fast localization of small substrate concentrations at small distances.

MMIP034

Marinobacterium sp. GAL22: A new model organism to study interactions between nitrogen-fixing bacteria and mangrove plants.

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Nitrogen fixing bacteria play a major role in re-mineralization processes in mangrove ecosystems. Most of the nitrogen in mangrove sediments is lost by denitrification and consequently is no longer available for metabolic processes in plants. Previous studies showed that nitrogen-fixing bacteria interact with mangrove roots making nitrogen available for plants. Although, nitrogen fixation is a very important process in mangrove ecosystems, very little is known about bacterial colonization strategies and physiological impacts on mangrove roots. The establishment of a model system consisting of a genetically accessible nitrogen-fixing bacterium and a mangrove plant is necessary to study the molecular mechanisms of this interaction. The aims of this study were to establish a nitrogen-fixing bacteria-mangrove model system, and to study the colonization pattern of the selected nitrogen-fixer on mangrove roots. For this, a selection of the bacterium was done according to the following criteria: firstly, the ability of the bacterial isolates to colonize and persist for more than one month on mangrove roots when inoculated with sediment-borne indigenous bacterial strains (fitness test). Secondly, their genetic accessibility had to be determined. To investigate the mangrove root colonization pattern of the nitrogen-fixing model bacterium, *Marinobacterium sp*. GAL22, which showed the strongest fitness and is genetically accessible, we used the *gus* reporter gene and a fluorescent protein to label the bacterium and followed by CLSM and light microscopy. Our results suggest that *Marinobacterium sp*. GAL22 could be used to establish a bacteria-mangrove model system to continue our investigation of the molecular mechanisms determining bacteria-mangrove interactions.

MMIP035

Impact of rising water temperature on bacterial activity and community compo-sition during a phytoplankton spring bloom in a mesocosm experiment

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Global warming and increasing water temperature are assumed to change the trophic interactions in aquatic food webs and might finally shift the system towards net heterotrophy. However, less is known how the specific activity and diversity of the dominating bacterial taxa are affected by increasing water temperature during phytoplankton spring blooms. Therefore we investigated the impact of different water temperature on the algae-bacteria coupling as well as the bacterial community composition during an indoor mesocosm experiment with natural spring plankton communities from the western Baltic Sea. An increase in water temperature of 6°C resulted, as predicted, in a tighter coupling between the diatom-dominated phytoplankton bloom and the heterotrophic bacterial community, accompanied by a strong increase in carbon flow from phyto- to bacterioplankton. The free-living bacterial community composition (BCC) during the phytoplankton bloom was analysed by 16S rRNA based clone libraries and DGGE fingerprints. Generally, the BCC was strongly influenced by the stage of the phytoplankton bloom. Interestingly, during the phytoplankton peak, the BCC was dominated by Gammaproteobacteria independent from temperature (93% cold and 51% warm). The BCC in the warm treatments was additional characterised by an increased appearance of Bacteroidetes (20%) and Betaproteobacteria (26%). Among the Gammaproteobacteria clones, the *Glaciecola* group was clearly dominant, representing 55% and 44% in the cold and warm treatments, respectively. A newly designed CARD-FISH probe (GC1205) verified this dominance, showing that up to 36% of the free-living bacteria corresponded to the *Glaciecola* group. However, abundances of *Glaciecola* developed synchronously to the phytoplankton spring bloom independent to the water temperature. These results show that phytoplankton blooms are

accompanied by highly adapted bacterial key players with only weak responses to temperature changes. Future studies have to show whether these also dominate the carbon flow from phyto- to bacterioplankton.

MMIP036

Strain TN10130, a diazotrophic, cellulolytic bacterial endosymbiont isolated from the gills of the wood-boring bivalve *Teredo navalis* LINNAEUS, 1758

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Wood is a rather hardly degradable nitrogen-deficient natural composite. Its degradation requires the presence of special enzymes to decompose the building blocks, e.g. cellulose, hemicellulose and lignin. In the terrestrial environment, fungi play an important role in wood deterioration, causing white, brown and soft rot. But also ruminants and insects like termites are able to degrade cellulosic materials with the help of symbiotic microorganisms (bacteria, protists) present in their guts and, in the latter case also by using host-encoded cellulases [5].
In the marine environment, almost all members of the family Teredinidae, shallow-water bivalves commonly known as shipworms, consume and bore into wood [1]. This ability is supported by the presence of a bacterial community that, instead of being present in the gut, lives in specialized cells (bacteriocytes) inside the gills of the host [4]. Besides supplying cellulolytic enzymes, these endosymbionts possess another intriguing feature limited to prokaryotic life, i.e. the fixation of molecular nitrogen [3].
Strain TN10130 was isolated from a shipworm specimen of *Teredo navalis* LINNAEUS, 1758 that was prepared from a pine board artificially exposed in the Western Baltic Sea (Eckernförde Harbor) for four weeks in the summer of 2009.
TN10130 is a γ-proteobacterium that shares the same basic features - diazotrophic growth and the degradation of cellulose - as the previously described strains of the culturable endosymbiont *Teredinibacter turnerae* T7902T [2], but comparative sequence analysis of the SSU rRNA gene as well as phenotypic and genotypic analyses suggest it to be a new taxon.

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MMIP037

Growth phase-dependent membrane lipid composition of the marine ammonia-oxidizing archaeon *Nitrosopumilus maritimus*

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Marine ammonia-oxidizing archaea (AOA) are a cosmopolitan and abundant group of microorganisms accounting for up to 20% of the bacterioplankton in the oceans. AOA play a key role in the marine nitrogen cycle by catalyzing the first and rate-limiting step of nitrification even at extremely low ammonia concentrations. The membrane lipids of AOA consist mainly of isoprenoidal glycerol dialkyl glycerol tetraethers (GDGTs). Intact polar GDGTs are widely used as biomarkers for occurrence and distribution of active AOA. In contrast, fossil core GDGTs originating from planktonic AOA are also found in sediments and frequently used as a paleoceanographic proxy (expressed in the TEX_{86} index) for reconstructing past surface water temperatures. Thus, understanding the influence of physiological and environmental parameters on membrane lipid composition in marine planktonic AOA is crucial to the evaluation of associated proxies and the application of lipid biomarkers in tracing archaeal distribution, seasonality, and activity in the marine water column. Here, we studied the membrane lipid composition of the first cultured marine AOA *Nitrosopumilus maritimus* in different growth stages. We analyzed core and intact polar lipids of *N. maritimus* harvested during early exponential, mid-exponential, late exponential, early stationary and late stationary growth phases. Changes in the relative abundances of characteristic intact polar GDGTs were monitored during growth to assess their potential as biomarkers for active AOA in environmental studies. Furthermore, we evaluated the impact of growth stage related changes in core GDGT composition on the TEX_{86} paleothermometer. Our results promote the understanding of isoprenoidal GDGTs as biomarkers for the activity of AOA in the marine water column and how this is reflected in the TEX_{86} paleotemperature proxy.

MMIP038

Proteomic and metabolic characterisation of the shipworm symbiont TN10130 during phosphate limitation

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The Gram-negative bacterial endosymbiont TN10130, isolated from the "naval shipworm" *Teredo navalis* LINNAEUS, 1758 is cultivable under laboratory conditions and thus conveniently accessible for genome sequencing and proteomic analysis. Its wood-boring bivalve host is known for its destructive way of living that especially affects marine structures at the south western Baltic Sea coast. Its nutrition is based upon the digestion of wood as well as on nitrogen fixation, both of which are facilitated by the bacterial symbionts. Genome pyrosequencing of the bacterial isolate TN10130 showed - as expected - the presence of genes encoding for enzymes involved in elemental nitrogen fixation as well as for polysaccharide degradation-related enzymes - particularly those for cellulose degradation. The genome analysis furthermore revealed sequences coding for polyketide synthases (PKSs), large protein clusters for the synthesis of bioactive compounds (polyketides). In this study, the symbiont's physiological stress response to phosphate limitation was investigated. For this purpose, the isolate TN10130 was cultivated in a special medium (shipworm basal medium, SBM) with high and low phosphate concentrations, respectively. Using proteome (1D- and 2D-gel based) and exometabolome approaches (1H-NMR spectroscopy), global changes in the protein composition and in the secretion profile of the isolate were monitored and compared between the control and the phosphate limitation condition. Our results indicate that a distinct physiological stress response to phosphate limitation is induced in the shipworm symbiont, which seems to include the enhanced production of the polyketide synthase proteins. Implications on the survival strategies of TN10130 - inside or outside its host - of this and other findings from this first-time proteomic and metabolomic study will be discussed.

MMIP039

Microbial ecology of free-living microorganisms in fluids of the Menez Gwen deep-sea hydrothermal field

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Deep-sea hydrothermal vents are unique environments featuring extreme conditions in terms of temperature, pressure and geochemical conditions. Nevertheless, they host highly productive and diverse ecosystems. Energy gain and primary production of these ecosystems are based on chemolithotrophy, giving rise to extensive microbial life. These processes are dependent on the chemical composition and physical features of the hydrothermal fluids. But also vice versa, the chemical composition of the emitted fluids itself can be influenced by e.g. subsurface microbial communities1. The so called "diffuse fluids", moderate enough for microorganisms to survive, are considered to be windows to microbial life in the subsurface2.
In our project we study the ecology of microbial communities in diffuse fluids of the Menez Gwen deep-sea hydrothermal field. The study aims at

the advancement of our knowledge of microbial communities populating hydrothermal fluids.
Samples for this interdisciplinary study were collected at several differently characterized diffuse venting sites employing a remotely operated vehicle (ROV). In a first step of our analyses, different DNA extraction methods were tested to find the most efficient protocol to extract metagenomic DNA for diversity analyses from membrane filters suitable also for low sample volumes. The results were evaluated by automated ribosomal RNA intergenic spacer analysis (ARISA). Subsequently, 16S rRNA pyrotags were generated for fluid samples from three different sites within the Menez Gwen hydrothermal field, using universal primers. The abundance of key microbial taxa was determined by fluorescence in situ hybridization. The results of these analyses will be presented together with fluid geochemical data determined during sampling in order to reveal the interaction of geosphere and biosphere at Menez Gwen.

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MMIP040

Light-enhanced survival of *Dinoroseobacter shibae* during longterm starvation

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Dinoroseobacter shibae is an aerobic anoxygenic phototroph (AAP), capable of using light as an additional energy source under oxic conditions (1-3). While light is known to increase growth yields in continuous cultures only marginally (2), we assumed that the cells benefit from light particularly under conditions of carbon and electron donor limitation. To test this, long-term starvation experiments were performed with cells grown in both complex marine broth and defined minimal medium with succinate as the only substrate. The cells were incubated without substrate supply over six months under three different conditions, i.e. continuous darkness, continuous light, and a day-night cycle (12 h/12 h). To assess viability and the energetic state, several biomass parameters (total and live count, dry mass, pigment and ATP content) and activities (respiration rates, light- and respiration-driven proton translocation) were analysed.
From the three different incubation conditions, the day- and night cycle supported longest survival of the cells. Cells starved under the day- and night cycle had tenfold higher total and viable counts and higher bacteriochlorophyll concentrations after six weeks of starvation. Respiration rates, proton translocation and ATP concentrations also showed significant differences between cells starved under continuous light and under the daily rhythm. Cells starved under continuous light did not show any activities after three weeks. The optimum light intensity was 12 μE m^{-2} s^{-1}, whereas 53 μE m^{-2} s^{-1} caused higher death rates than dark incubation. Cells grown in complex medium survived longer than those grown with succinate. Our experiments show that *D. shibae* clearly benefits from the day- and night cycle during starvation.

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MMIP041

Combination of fluorescence lectin-binding analysis (FLBA) and CARD-FISH for simultaneous detection of marine *Bacteroidetes* and lectin-specific EPS glycoconjugates

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Bacteria are able to produce a variety of EPS compounds having different biochemical composition. EPS glycoconjugates in form of capsules or slimes are involved in attachment, biofilm and bioaggregate formation in freshwater and marine habitats. The characterisation of glycoconjugates by fluorescently labelled lectins in combination with CARD-FISH gives the opportunity to directly link the identity of a specific bacterial group with the identity of glycoconjugates which may have a role in aggregate formation. Fluorescently labelled lectins are able to stain carbohydrate structures according to their specificity. The present study was undertaken to characterise *in situ* whether *Bacteroidetes* produce EPS glycoconjugates in form of capsules or slime. Therefore, 77 lectins were screened on >10 μm fractions retrieved from Helgoland waters, during the phytoplankton spring bloom 2011. From these 77 lectins, 12 stained the EPS matrix of the aggregates. Whereas 9 of them were able to stain the cell surface of either *Bacteroidetes* or unknown bacteria, the other 3 lectins stained the cell surface of phytoplankton cells, in particular their cell extensions (*setae*). It was also observed that *Bacteroidetes,* especially of the *Polaribacter* clade, attached at high density to the diatom *Chaetoceros* spp. Bacteria attached not only to the cell surface, but also to the *setae*. In summary, the combination of CARD-FISH and Fluorescence Lectin-Binding Analysis (FLBA) revealed not only EPS glycoconjugate production of *Bacteroidetes*, it also visualised distinct niches for attachment and microhabitat formation.

MMIP042

Turn on the lights: Benefits of aerobic anoxygenic photosynthesis for the *Roseobacter* clade affiliated (RCA) cluster

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Photoheterotrophy is a surprisingly common feature among the main bacterioplankton groups. Within Alphaproteobacteria quite a few representatives of the *Roseobacter* clade are known to carry genes encoding bacteriochlorophyll a (BChla) and thus are capable of aerobic anoxygenic photosynthesis (AAnP). However, still little is known about the genomic organisation and expression of these genes in the major bacterioplankton groups including the *Roseobacter* Clade Affiliated (RCA) cluster and, more importantly, about potential benefits of this complementary energy source. Genomic analyses of the RCA type strain Candidatus *Planktomarina temperata* revealed the presence of the entire BChla operon. The RCA specific photosynthetic operon was present in different metagenomic libraries of other studies, from a Norwegian fjord, the western English Channel, the western coastal Atlantic and the eastern and western Pacific. During a metatranscriptomic study of environmental samples in the German Bight (North Sea) we were able to show the expression of the RCA photosynthetic operon, pointing to the biosynthesis of BChla in situ and thus to generating energy via AAnP by RCA microbes. To examine more specifically effects of AAnP on the growth of Cand. *P. temperata* we carried out growth experiments in a light-dark cycle versus dark conditions. During the exponential growth phase there was no difference between both conditions. However, in the stationary phase, cell numbers grown in the light-dark cycle remained twice as high as in the dark and the cell size and its granularity of the former was significantly lower compared to the dark. First results of RT-PCR analysis indicated the expression of photosynthetic pufM genes for Cand. *P. temperata* during these experiments.

MMIP043

Gammaproteobacteria are the major consumers of acetate in tidal sediment

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Up to 50% of organic matter (OM) in organically-rich continental shelves is degraded in sediments. Acetate is a key intermediate in OM degradation and is used as carbon and energy source by many microbes. To identify acetate-consuming microorganisms, previous studies exclusively relied on non-quantitative approaches such as stable isotope-probing (SIP) that biases towards organisms assimilating the ^{13}C-labelled substrate into nucleic acids after rather long-term incubations. Quantitative data on the total and relative contribution to acetate turnover by distinct microbial populations in environmental samples are generally missing. In this study we quantified assimilation of isotopically-labelled acetate by different bacterial populations in intertidal sediments at the single cell and population level. We followed assimilation of ^{14}C -labelled acetate under oxic and anoxic conditions using microautoradiography combined with fluorescence in situ hybridization (MAR-FISH). Under oxic conditions only 12-17% of total cells assimilated acetate, in contrast to 6 to 10% under anoxic conditions. The relative abundance of MAR-positive cells sharply decreased with depth, displaying only 2% in the deepest sediment layer of 9-10 cm. Gammaproteobacteria were the dominant acetate assimilating organisms and

accounted for up to 25 and 67% of all MAR-positive cells under all conditions tested, although they accounted only for 20-30% of the microbial community. Roseobacter-related bacteria accounted for 3-8% of acetate assimilating cells. When anoxic sediment was incubated with oxygen, numbers of MAR-positive cells increased to abundances similar to those from oxic sediment layers. Unexpectedly, sulfate-reducing bacteria (SRB) of the Desulfobacteraceae did not show significant assimilation of acetate. NanoSIMS re-confirmed uptake of ^{13}C-acetate by single gammaproteobacterial and Roseobacter-cells. Using flow sorting of FISH-stained cells from ^{14}C-acetate incubations we could determine the bulk uptake of ^{14}C-acetate in individual populations, which revealed a cell-specific uptake rate by gammaproteobacteria of 0.4-1 fmol C cell^{-1} 12h^{-1} under oxic conditions. Our data show that gammaproteobacteria account for the major fraction of acetate assimilation that was previously overlooked due to methodological limitations in SIP studies.

MMIP044

Highly similar methanotrophic endosymbionts shared by deep-sea mussels of different clades, geographic regions and habitats

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Bathymodiolin mussels are found worldwide in the deep-sea in habitats rich in reduced inorganic compounds such as hydrothermal vents and hydrocarbon seeps. The mussels live in symbiosis with chemosynthetic bacteria that gain energy from oxidizing methane, or reduced sulfur compounds and hydrogen, and assimilate inorganic carbon and methane into organic compounds that are passed on to the host for its nutrition. Bathymodiolin mussels can be associated with gammaproteobacterial methane oxidizers, others harbor gammaproteobacterial sulfur oxidizers, and a number of species host both symbiont types. Most host species are bound to a specific habitat which is assumed to depend on their symbiont type and adequate substrate availability. All mussels analyzed to date harbor closely related but distinct 16S rRNA symbiont phylotypes, with the exception of closely related mussel species from hydrothermal vents on the Mid-Atlantic Ridge.
To resolve the phylogenetic relationships and biogeography of the symbionts of bathymodiolin mussels in more detail we analyzed mussels from hydrothermal vents on the Mid-Atlantic Ridge, hydrocarbon seeps in the Gulf of Mexico and an undescribed bathymodiolin species from seeps in the northern Indian Ocean. For reconstruction of the host phylogeny we analyzed the mitochondrial cytochrome oxidase subunit I gene (COI). The phylogeny of the bacterial symbionts was based on analysis of their 16S rRNA gene and the gene for the bacterial DNA recombination protein RecA. Our molecular data indicate highly similar methanotrophic endosymbionts shared by mussels from different host clades, geographic regions and habitats. This is particularly interesting because bathymodiolin hosts acquire their symbionts from the environment. It sheds new light on the dispersal capabilities and biogeography of free living symbionts as well as the host-symbiont specificity.

MMIP045

Diffusion growth chambers for the isolation of novel sponge-associated bacteria

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Marine sponges and their symbiotic bacteria have been shown to be a rich source of biologically active secondary metabolites. One problem in studying the chemistry of these microorganisms is the estimation that only about 1% can be cultivated. Our goal was to develop a method for the isolation of sponge-associated bacteria by simulating the microorganisms' natural environment. Diffusion Growth chambers (DGCs) were designed to allow incubation of a sponge-bacteria agar mix inside the sponge tissue. Chambers had 0.22µm filters on either side to allow the exchange of nutrients and sponge secondary metabolites while at the same time retaining the inoculated bacteria mix inside. We predicted that this method would allow the isolation of new sponge bacteria, to facilitate the study of their secondary metabolites. Diffusion chambers were inoculated with tissue of the sponge Rhabdastrella globostellata, collected in Apra Harbor, Guam. The chambers were incubated inside this sponge in the field for periods of 4 weeks. The chambers were then collected and the contents diluted and plated onto different media (Marine Broth, M8 and Actinomycetes Isolation Agar). Part of the bacterial agar mix was used to reinnoculate new media, which then was pipetted into new chambers and again incubated inside the sponge for 4 weeks. We repeated this inoculation-incubation approach a total of 4 times (16 weeks). Selected colony forming units (CFUs) were transferred repeatedly until pure cultures were obtained. Analysis of 16S rDNA sequence data revealed that numerous of the isolated bacterial strains were either previously uncultured or were less than 97% similar to other cultured strains, indicating that the method is indeed a promising approach for the culture of novel sponge-associated bacteria.

MMIP046

Distributions and diversity of bacteria of the class *Dehalococcoidetes* (phylum *Chloroflexi*) in the marine subsurface

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Bacteria of the class *Dehalococcoidetes* (DEH) (phylum *Chloroflexi*) are widely distributed in the subsurface and are especially prevalent in the deep marine subsurface. Nevertheless, very little is known about the distributions of DEH, the extent of DEH diversity and the variability of DEH sub-group distributions at different sites and depths. Therefore, this study specifically examined the distribution and diversity of DEH through depths of various marine sediment cores by quantitative PCR and pyrosequencing using 16S rRNA gene targeting primers. Quantification of DEH showed populations establish in shallow sediments (i.e., upper centimeters) although often as low relative proportions of total *Bacteria*, yet often became more prevalent in deeper sediments. Pyrosequencing revealed pronounced diversity spanning many DEH sub-groups can co-exist within single biogeochemical zones and appears to be related to total organic matter content. In some cases, the low relative proportions of DEH in shallow sediments constitute 'rare biosphere' populations, yet the specific detection method enabled deep insights into DEH diversity that might normally be missed by general microbial community surveys in such highly diverse marine sediment environments. The pyrosequencing analyses also revealed that clear shifts in the relative proportions of DEH sub-groups occur with depth in various marine cores. These changes are related to biogeochemical factors including the availability of electron acceptors such as sulfate, and also likely due to the quality of organic matter. Additionally, a general trend among the cores in which DEH shifted away from broadly related clades to other broadly related clades with increasing depth, suggests some wider evolutionary relationships among major DEH clades with greater abilities to survive in deep sedimentary conditions. Collectively, the results provide important baseline ecological data regarding the distributions of DEH and DEH sub-groups, and suggests that the DEH exhibit a much wider physiological, biochemical and genomic diversity than previously recognized. This diversity therefore likely contributes to their widespread distributions in various biogeochemical zones in the marine subsurface.

MMIP047

Functional characterization of polysaccharide utilization loci in the marine Bacteroidetes 'Gramella forsetii'

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The ability for biopolymer decomposition enables *Bacteroidetes* species to assume a dominant role in contrasting ecological niches such as the marine realm, freshwater, soils and the human intestine. Recent metaproteomic studies of coastal surface waters demonstrated that abundance of this phylum is correlated with the release of complex organic matter after algae blooms entailing pronounced increases in microbial TonB-dependent transporters and carbohydrate-active enzymes. Dynamic succession on low taxonomic levels reflected also a highly varying nutrient supply, which in turn implies a broad diversity within the relevant protein groups. To elucidate the specificity of distinct protein classes for selected algae-derived carbon sources, we introduced an *in vivo* N^{15} isotope labeling approach to cultures of the North Sea *Bacteroidetes* 'Gramella forsetii'. Profiles of its labeled proteome revealed a large number of polysaccharide utilization loci

(PULs), particularly located in the membrane subfraction, and allowed us to gain a more precise idea of single metabolic processes involving polymer degradation in marine coastal systems. Additionally, upregulation of several surface-associated hypothetical proteins provided indications for potential substrate-dependent functions that might contribute to a *Bacteroidetes*-specific response in nutrient-rich environments.

MMIP048

Microbial response to temperature induced anoxia in a marine carst lake

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Lake Rogoznica is a small, marine, carst lake on the east Adriatic coast. Its water column is stratified from winter to late summer, with an oxygenated top layer and an anoxic, sulfidic bottom layer (Burić Nakić et al., 2009). At the layer boundary (~10m), anoxygenic phototrophs have been observed. In autumn stratification is disrupted by a mixing event, usually oxygenating the entire water column. However, in warm years, such as 1997 or 2011, oxygen solubility in lake waters is low and mixing leads to oxygen depletion throughout the water column, accompanied by the formation of colloidal S^0. Such anoxic events lead to mass mortality of plankton and nekton species (Ciglenečki et al., 2005) and cause an evident shifts in the lakes biocenosis.
We used culture independent methods (16S rRNA and *soxB* gene sequencing and CARD-FISH) to monitor the lake microbial community prior to, during and after the anoxia event in autumn 2011.
During stratification, higher cell numbers were detected in deeper layers (>$5x10^6$ cells/mL) than at the lakes surface (<$5x10^6$ cells/mL). The community in the oxygenated layer was dominated by *Cyanobacteria* and *α-Proteobacteria* of the SAR11 clade, while in the bottom layer *δ-Proteobacteria* were detected. The anoxygenic phototrophs at the chemocline are uncultured *Chlorobi*, related to *Chlorobium phaeovibrioides*. During the mixing event, cell numbers generally increased and a drastic change in microbial community composition was recorded. *γ-Proteobacteria* of the SUP05 cluster dominated the entire, chemically homogeneous water column. Presence of SUP05 related *soxB* genes implies a biotic origin of colloidal S^0, as reported for a SUP05 isolate (Marshall and Morris, 2012) and as previously recorded in pelagic oxygen minimum zones (Lavike et al., 2009). After reestablishment of stratification, the initial microbial communities in the oxygenated layer, *Chlorobi* chemocline layer and sulfidic layer are restored.
The microbial community composition of lake Rogoznica with its sensitive ties to water chemistry, most of all to temperature dependent oxygen solubility, highlights the large effect of environmental change on aqueous environments. Further, temperature increase could lead to more frequent anoxic events, effecting not only microbial communities, but all dependent organisms.

MMAV001

High resolution typing of livestock-associated methicillin-resistant *Staphylococcus aureus* using whole genome mapping enables identification of transmission events

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Question: Typing of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) using current typing methods yields insufficient discriminatory power to enable identification of transmission events caused by this clade. Here, we evaluated the capability of a technique called whole genome mapping (WGM) to type LA-MRSA and elucidate transmission events.
Methods: LA-MRSA and MRSA isolates obtained from outbreaks and non-related isolates were typed by WGM, spa-typing, and PFGE (n=78). In addition, isolates cultured from 17 veterinarians and their family members were used to study transmission of LA-MRSA. After extraction, high molecular weight DNA (>250 kb) was transferred into micro-channels in which the DNA molecules were stretched, immobilized, digested with *Afl*II and fluorescently stained within the micro-fluidic system. The resulting restriction fragments were photographed and sized in the Argus™ machine and assembled into ordered, high-resolution restriction maps which were imported into the BioNumerics software for analyses.
Results: Repetitive analysis of the same DNA sample on 5 consecutive days and of DNA obtained from isolates that were sub-cultured for 30 days revealed a 99% similarity between maps, showing highly reproducible results. Of three previously described outbreaks two (LA-MRSA and MRSA) were confirmed, but WGM revealed major differences between the maps of the third, indicating not all isolates belonged to this outbreak. Analysis of LA-MRSA isolates obtained from 17 veterinarians yielded 13 different maps whereas only 5 spa-types were found among these isolates, indicating the high discriminatory power of WGM. LA-MRSA isolates obtained from 2 veterinarians and their family members were further investigated. WGMs of one family were nearly indistinguishable, indicating that transmission between veterinarian and its family had occurred. In contrast, the maps of the LA-MRSA from the other veterinarian and his household differed considerably from each other, demonstrating colonization had not occurred due to transmission.
Conclusion: Until now, PFGE was the best method to differentiate LA-MRSA isolates. However, whole genome mapping is a more discriminatory and a more reproducible technique to investigate possible transmission events caused by LA-MRSA.

MMAV002

Host-pathogen interactions during a catheter-associated urinary tract infection analysed by metaproteomics

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Long-term catheterization of the bladder leads inevitably to a mostly asymptomatic bacteriuria. So far, only little is known about the bacterial defence strategies in the urinary tract and the human immune reaction in response to the bacterial biofilm. To investigate host-pathogen interactions, we analysed biofilms and urine from long-term catheterized patients by a semi-quantitative metaproteomic approach (1D-PAGE followed by LC-ESI-MS/MS). The phylogenetic assignment of the identified proteins revealed that the predominant colonizer of one of the investigated biofilms was *Pseudomonas aeruginosa*, followed by *Morganella morganii* and the obligate anaerobic *Bacteriodetes* sp. The functional assignment of proteins suggests specific adaptation strategies of the biofilm inhabitants. First, due to the presence of *Bacteriodetes* sp. and the identification of *P. aeruginosa* proteins involved in denitrification, known to take place under anaerobic conditions, biofilms in the urinary tract can be considered as a microaerobic environment. Second, *P. aeruginosa* expresses several factors to overcome iron-limitation in the bladder. Last several bacterial proteases, which were exclusively identified in the catheter-associated urine, appear to participate in immune evasion of *P. aeruginosa*. Interestingly, among the identified human proteins were various major players of the innate immune response, including proteins originating from neutrophil azurophilic and specific granules as well as complement compounds and complement regulatory proteins, most abundant were complement compound 3, factor B, and factor H. A comparison of the urinary proteome of patients and healthy test persons demonstrated that proteins of the innate immune system are indeed enriched in urine of the catheterized persons. In conclusion, our study contributes to a better understanding of host-microbe interactions related to asymptomatic urinary tract infections and helps to unravel specific adaptation mechanisms employed by the opportunistic pathogens to adapt to the bladder environment.

MMAV003

Role of the proposed *Helicobacter pylori* energy sensor TlpD *in vivo* in the Mongolian gerbil model and whole genome analysis of a gerbil-adapted *H. pylori* strain

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The human gastric pathogen *Helicobacter pylori* requires flagellar motility and taxis in infection and persistence. *H. pylori* encodes for four homologs to chemotaxis receptor proteins (MCPs). Three, TlpA, TlpB, and TlpC, function as transmembrane receptors, while the fourth MCP, TlpD, lacks a transmembrane domain. We identified this soluble receptor to be a novel type of cytoplasmic taxis sensor, which has a proposed role in energy- and metabolism-dependent taxis (Schweinitzer et al., 2008). We suggested that energy taxis is dominant over other modes of taxis in *H. pylori in vitro* and *in vivo*. However, previous *in vivo* colonization data of a *H. pylo*ri TlpD mutant in a mouse model (Williams et al., 2007) indicated that TlpD may not be essential *in vivo*. In addition, the mechanism of TlpD function remained unclear. We therefore wanted to investigate the role of TlpD *in vivo* in a gerbil infection model. This approach included whole genome analyses and comparisons of gerbil-adapted *H. pylori* strains.
We have investigated the role of TlpD in a Mongolian gerbil infection model, which closely mimics the human gastric physiology, including a very low pH in the gastric lumen. A gerbil-adapted *H. pylori* strain, HP87 G7, was able to perform TlpD-dependent energy behavior, while its isogenic mutant in *tlpD* had lost it. A complemented strain regained the ability. Gerbil infections demonstrated that TlpD was essential for the initial colonization in the antrum as well as for persistence. A long-term effect of TlpD deficiency in reducing *H. pylori* colonization was observed both in the antrum and corpus region of the gerbil stomach. The complete genome sequence of the gerbil-adapted strain HP87 P7 was elucidated and compared with HP87 pre-gerbil, with a HP87 P7 *tlpD-* clone pre-gerbil and with a reisolate pool of HP87 P7 *tlpD-* which persisted during gerbil infection. Differences of the strains after gerbil adaptation were observed but no major losses or gains of genes or gene functions. The results indicate that TlpD is strongly involved in *H. pylori* colonization and has a decisive function during initial colonization, but also during bacterial persistence.

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MMAV004

Whole-transcriptome analysis of Staphylococcus aureus under laboratory and infection-related conditions

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High-density array formats and next generation sequencing (NGS) technologies have opened the way to the characterization of entire transcriptomes by genomic tiling arrays and RNA-seq techniques. Such whole-transcriptome studies have revealed an unexpectedly high level of complexity of bacterial transcriptomes. We have recently analyzed the transcriptome of the Gram-positive model bacterium *Bacillus subtilis* exposed to a wide range of nutritional and environmental conditions [1]. This study has established one of the most comprehensive repertoires of transcription units in a prokaryote, including the discovery of more than 500 putative novel genes and abundant antisense RNAs. By de novo genome-wide identification of the sigma factor regulons we have for the first time quantified the contribution of a bacterium's alternative sigma factors to transcriptome plasticity.

Here we report a systematic and quantitative investigation of the transcriptome of the Gram-positive pathogen *Staphylococcus aureus,* known as both, a human commensal and a versatile pathogen causing a variety of diseases ranging from skin infections to systemic diseases. To elucidate the adaptive potential of *S. aureus* on the level of transcription, we have analyzed the responses of *S. aureus* HG001, a derivative of NCTC8325, to different *in vitro* growth conditions and upon interaction with host cells by means of high-density tiling array hybridizations. While focusing on the responses of *S. aureus* to clinically relevant antibiotics and to internalization by eukaryotic cells - applying cell culture infection models with human S9 bronchial epithelial cells and THP1 macrophages, respectively - our study also involves conditions like growth in various media or human serum and adaptation to nutrient and oxygen limitations. Based on this compendium of strand-specific transcription profiles we have performed comprehensive analyses including a systematic mapping of transcription units and the annotation of novel transcripts, i.e. putative new CDSs, putative regulatory RNAs, antisense RNAs, 5'- and 3'-UTRs. Importantly, by combining the expression profiles across the conditions with transcription factor binding motif searches, the data also facilitate deeper insights into the transcriptional regulatory network of *S. aureus*.

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MMAV005

Impact of a putative carboxypeptidase lipoprotein SPD_0549 on pneumococcal peptidoglycan synthesis and virulence

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Streptococcus pneumoniae naturally reside in the human oral cavity but cause also serious local and invasive infections including otitis media, pneumonia, septicemia, and meningitis. Lipoproteins are involved in various processes such as nutrient uptake, colonization, and protein folding. Hence, lipoproteins represent a class of surface proteins indispensable for bacterial fitness and virulence. The bioinformatic analysis of the genome of strain D39 revealed the presence of 37 genes encoding lipoproteins. Here we introduce the SPD_0549 lipoprotein, which is annotated as hypothetical lipoprotein without assigned function in D39 and TIGR4 pneumococcal strains. Pneumococcal mutants deficient in lipoprotein SPD_0549 were generated in *S. pneumoniae* by insertion deletion mutagenesis. The mutants were characterized and compared with the wild-type strain by standard molecular techniques and immunoblot analysis which indicated loss-of-function of the lipoprotein. Under *in vitro* conditions using complex and chemically defined media the lipoprotein mutants showed only a slight growth delay compared to the isogenic wild-type. Flow cytometry and immunofluorescence microscopy experiments confirmed surface location of the SPD_0549 lipoprotein for the wild-type and indicated the absence on the lipoprotein mutant. The effect of the lipoprotein deficiency on phagocytosis was tested using macrophages, while the impact on adherence was investigated using A549 epithelial cells. Phagocytosis of the mutants was significantly affected as shown by the antibiotic protection assay and double immunofluorescence microscopy. The acute pneumonia mouse infection model and real-time bioimaging demonstrated a substantial attenuation of the *spd_0549* mutant compared to its isogenic D39 wild-type. Additionally, to identify the DD-carboxypeptidase activity of SPD_0549, fluorometric OPTA method and HPLC were employed. Taken together, the results suggest that the putative carboxypeptidase lipoprotein SPD_0549 may play a role in peptidoglycan synthesis and that loss-of-function affects virulence.

MMAV006

Haemophilus influenzae surface fibril (Hsf) is a double folded trimeric autotransporter that binds two vitronectin molecules to enhance serum resistance

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Haemophilus influenzae type b (Hib) is a human respiratory pathogen that causes meningitis, pneumonia and septicemia in children. This pathogen is frequently resistant to the host innate immune system (complement-mediated killing), which is mediated by recruitment of complement

regulators such as vitronectin, C4b binding protein (C4BP) and Factor H at the surface of bacteria resulting in inhibition of membrane attack complex (MAC) formation. Vitronectin (Vn) inhibits the formation of the MAC by inhibiting C5b-C7 complex formation and C9 polymerization. In this study, we reported that Hib acquires Vn at the surface by using Hsf. Hsf is a high molecular weight (750 kDa) trimeric autotransporter, which is located at the surface of bacteria as confirmed by using flow cytometry and Transmission Electron Microscopy (TEM). The amino acid sequence of Hsf has several repetitive domains that was analyzed by using antibody mapping and TEM. On the basis of a partial known structure and our experimental data, the full structure of Hsf was modeled. The protein appeared in a double folded structure at the surface of Hib, and Hsf simultaneously binds two Vn molecules. Vn-binding domains were identified by studies with truncated proteins. Expression of full length Hsf in a heterologous host acquired Vn binding similarly to Hib and showed a marked increased serum resistance and an inhibited MAC formation at the bacterial surface. In addition, we mapped the Hsf recognition site in the Vn molecule and found that amino acids 353-374 were important. Taken together, the fine tuned protein-protein interaction showed a mechanism of an advanced level of protection shielding bacteria from the innate immune response, and thus significantly increased survival of Hib in serum.

MMAV007

Crystal structure and functional mechanism of the human antimicrobial membrane channel dermcidin

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Multicellular organisms fend off bacterial and fungal infections by peptide-derived broad-spectrum antibiotics. The human body provides an extensive surface for the growth of microbes and their suppression requires the secretion of efficient antimicrobial peptides (AMPs). As AMPs directly compromise the integrity of microbial cell membranes and thereby evade many pathways of resistance development, they are envisioned as a foundation for the next generation of antibiotics. However, despite the identification of a large number of AMPs, the mechanistic and structural basis of their antibiotic action has remained speculative. Here, we present the oligomeric channel structure and antibiotic mechanism of dermcidin (DCD), a major human AMP produced in sweat glands and expressed on the human epidermal surface. The X-ray crystal structure, functional data and atomistic simulations of DCD in membranes reveal that its active state is a transmembrane barrel-stave channel, constructed from a hexamer of anti-parallel, elongated helices stabilised by zinc ions. Electrophysiological measurements of the channel in planar lipid bilayers show that DCD forms highly conductive pores that are regulated by zinc. Molecular dynamics simulations in lipid bilayers provide detailed insights into the permeation mechanism for ions and water, and demonstrate an adjustment of the pore to various membrane types. Our study thus provides a comprehensive mechanism for the membrane-disruptive action of a mammalian AMP at the atomistic level. We anticipate these results to form the basis for the structure-based, rational design of new AMP-derived antibiotic agents. As DCD is active against multi-resistant ***S. aureus*** and ***C. albicans*** strains, rationally altered DCD variants may have an even enhanced potential to treat bacterial infections that are no longer susceptible to traditional antibiotics.

MMAV008

Unique staphylococcus aureus wall teichoic acid prevents phage-mediated horizontal transfer of pathogenicity Islands

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Staphylococcal virulence genes are often strain-specific and carried by phages or phage related pathogenicity islands (PRPI) such as the *Staphyolococcus aureus* pathogenicity islands (SaPIs). It is generally assumed that these virulence genes have disseminated among the *S. aureus* population by-phage mediated transduction. SaPIs are packed with the help of structural proteins of resident helper phages to form infectious particles leading to SaPI transduction at very high frequency. While the discovery of high frequency SaPI transduction helps to understand the dissemination of important PRPI-encoded virulence factors such the toxic shock syndrome toxin hardly anything is known about the molecular routes of this specialized transduction. We recently verified that *S. aureus* wall teichoic acid (WTA) glycoepitopes serve as a receptor for lysogenic staphylococcal siphovirus such as Φ11 and Φ80α which were also known as helper phages involved in SaPI transduction. However, some *S. aureus*-infecting phages are known to have a narrow host range infecting only a few *S. aureus* strains. We focused on a specific clinical pneumonia isolate (VXP44) that is not infected by phages Φ11 and Φ80α and analyzed its WTA structure, which turned out to be unique. By using a whole-genome sequencing approach we identified and characterized the gene clusters involved in the biosynthesis of this special WTA type. Of note, strain VXP44 was found to be phyolgenetically isolated compared to all other sequenced *S. aureus* genomes most probable because of its unique WTA blocking horizontal gene transfer. Indeed, by expressing plasmid-encoded gene clusters responsible for biosynthesis of the standard polyribitol WTA type isolate VXP44 became susceptible to Φ11 and Φ80α and could be transduced with SaPI particles at high frequency indicating that WTA structure determines the capacity of *S. aureus* strains to participate in lateral gene transfer e.g. of PRPIs thereby contributing to the evolution of new clonal lineages.

MMAV009

Rapid and efficient compensation of low-fitness mutants resistant to several clinically important antibiotics

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The emergence and spread of antibiotic resistances has lead to the loss of many therapeutic options and represents a major public health concern. The molecular mechanisms of resistances often impose severe fitness costs to the resistant bacterial clones. The success of resistance mechanisms is strongly dependent on their influence on growth and survival, and it is therefore of importance to understand which factors ameliorate the fitness burden and increase the stability of antibiotic resistance mechanisms.
Using long-term evolution experiments we were able to minimize the associated fitness cost of several resistance-causing mutations to four clinically important antibiotics: Ertapenem, meropenem, fosfomycin and colistin. The resistance mechanisms include alterations in transport functions and two-component systems. By periodical screening of growth rates and resistance levels we determined the rate of compensation. In addition, we measured the correlation between bacterial fitness and susceptibility to these antibiotics.
Our results show that compensation of fitness costs is very rapid and efficient. While the compensation for constructed knockout strains with defects in porins (Δ*ompC*Δ*ompF* and Δ*ompR)* was only partial, compensated fosfomycin resistant strains (*uhpT*, *glpT*) increased their growth rate above wild type levels. Additionally, some of the evolved strains were able to tolerate even higher concentrations of the corresponding antibiotic than the unevolved parental strain.
These findings demonstrate the high adaptability and competitiveness of resistant mutants and underline the importance of preventing the initial development of antibiotic resistances.

MMAV010

Carbon source dependent hydrogen peroxide production in Mycoplasma species

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Mycoplasmas are gram positive pathogenic bacteria with a minimal genome. The virulence of *Mycoplasma pneumoniae* and *Mycoplasma genitalium* is based on two factors: their ability to attach to host cells and the production of hydrogen peroxide. This small cytotoxic molecule is produced dependent on the available carbon source (1). The current model for *M. pneumoniae* suggests that glycerol and glycerophosphatidylcholine can be taken up and converted to glycerol-3-phosphate, which is oxidized to dihydroxyacetone phosphate and hydrogen peroxide (2). In contrast, *M. genitalium* is also able to produce this toxin when glucose is used as single carbon source. Previous studies have shown that the gene product of *glpD* is responsible for hydrogen peroxide production in *M. pneumoniae* (3). A *glpD* mutant mutant of *M. genitalium* was still able to produce hydrogen peroxide with glucose but not with glycerol in the assay. Expression of the *M. genitalium glpD*

gene in a *M. pneumoniae glpD* mutant did not result in the production of hydrogen peroxide when glucose was added. We therefore conclude that there has to be a second as yet unknown enzyme in *M. genitalium* capable of peroxide production. To test this hypothesis, several potential genes were overexpressed and used for *in vitro* experiments, but none of the most promising candidates showed peroxide producing activity.
Furthermore we isolated *M. pneumoniae* mutants that are unable to produce hydrogen peroxide. Surprisingly the toxin can be produced in these mutants when glycerol and glucose or fructose were added. In these mutants the *glpD* gene was not disrupted, therefore the current model needs to be extended by a carbon source dependent glycerol utilization.

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MMAV011

Real-time *in vivo* imaging of invasive- and biomaterial-associated bacterial infections using fluorescently labeled vancomycin

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Question: Invasive and biomaterial-associated infections in humans are often difficult to diagnose and treat. Guided by recent advances in clinically relevant optical imaging technologies, we have explored the use of fluorescently labeled vancomycin (vanco-800CW) to specifically target and detect infections caused by Gram-positive bacteria.
Methods: The application potential of vanco-800CW for real-time *in vivo* imaging of bacterial infections was assessed in a mouse myositis model and a human *post-mortem* implant model.
Results: We show that vanco-800CW can: i) specifically detect Gram-positive bacterial infections in our mouse myositis model, ii) discriminate bacterial infections from sterile inflammation *in vivo*, and iii) detect biomaterial-associated infections in the lower leg of a human cadaver.
Conclusions: We conclude that vanco-800CW has a high potential for enhanced non-invasive diagnosis of infections with Gram-positive bacteria, and is a promising candidate for early phase clinical trials.

MMAV012

Role of QceB/C (FirR/S) in biofilm formation of non-typeable *Haemophilus influenzae* (NTHi)

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Non-typeable *Haemophilus influenzae* (NTHi) is a common pathogen associated with diseases such as acute otitis media or exacerbations in patients with chronic obstructive pulmonary disease. Biofilm formation is generally considered as one of the major virulence properties of NTHi enabling persistence during chronic diseases[1]. Several aspects of NTHi biofilms like sialic acid metabolism or extracellular DNA and proteins have been analyzed[2-4]. But, besides quorum sensing mediated by LuxS and its product autoinducer-2 (AI-2), general regulatory mechanisms are not completely elucidated[5]. In this study, we present data showing the involvement of the two-component signalling system QseB/C in biofilm formation of NTHi. For this an isogenic NTHi mutant of *qseC*, Hi3655KR2, was generated and tested for its *in vitro* biofilm forming capacity using crystal violet and live-dead staining. Hi3655KR2 showed a significant decrease in biofilm formation after 24 h of growth under static and semi-static conditions, and after 48 h under constant flow conditions. The production of AI-2 was not impaired in the mutant. Hence, here we suggest for the first time a regulatory circuit in NTHi, which controls biofilm formation by mechanisms other than or in addition to *luxS*-dependent factors.

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MMAV013

Campylobacter jejuni induces acute enterocolitis in gnotobiotic IL-10$^{-/-}$ mice via Toll-like-receptor-2 and -4 signaling

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Background: *Campylobacter jejuni* is a leading cause of foodborne bacterial enterocolitis worldwide. Investigations of the immunopathology in human campylobacteriosis are hampered by a lack of suitable vertebrate models. Mice display strong colonization resistance against the pathogen due to their host specific gut microbiota composition. We have recently shown that colonization resistance can be overcome in gnotobiotic mice as well as conventional IL-10 deficient animals with chronic colitis, but *C. jejuni* induced symptoms were rather subtle.
Methodology/Principal Findings: We generated gnotobiotic IL-10-/- mice by quintuple antibiotic treatment starting right after weaning, thereby preventing animals from commensal bacteria induced colitis. Following oral infection, *C. jejuni* B2 colonized the gastrointestinal tract of gnotobiotic IL-10-/- mice at high levels and induced acute enterocolitis within 7 days as indicated by bloody diarrhea and pronounced histopathological changes of the colonic mucosa. Immunopathology was further characterized by increased numbers of apoptotic cells, T- and B-lymphocytes as well as regulatory T-cells as well as elevated TNF-a, IFN-g, and MCP-1 concentrations in the inflamed colon. Infection of gnotobiotic IL-10-/- mice with a commensal *E. coli* strain, however, did not induce disease indicating a *C. jejuni* specific induction of acute enterocolitis. *C. jejuni* B2 infection of gnotobiotic IL-10-/- mice additionally lacking Toll like receptor (TLR) -4 or -2 revealed that immunopathology is mediated by TLR-4- and, less distinctly, by TLR-2 dependent signalling of *C. jejuni*-LPS and -lipoprotein, respectively.
Conclusion/Significance: We here present a novel murine *C. jejuni* infection model displaying acute enterocolitis and thus mimicking severe episodes of human campylobacteriosis. This acute model proves useful for further dissecting the immunopathological mechanisms underlying *Campylobacter* infections *in vivo* and to elucidate the interplay between intestinal pathogens, the commensal intestinal microbiota and the innate as well as adaptive immune system of the host.

MMAV014

Multiresistant uropathogenic *Myroides* sp. with unusual morphology features

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Myroides odoratimimus and *Myroides odoratus* (formerly *Flavobacterium odoratum*) are Gram negative rods with a characteristic fruity odor. They have a strictly respiratory metabolism with oxygen as terminal electron acceptor. Both species were frequently isolated from clinical specimen although they do not belong to the human microflora. Several studies mention *Myroides* spp. in the context of septic shock, pneumonia, cholecystitis and soft tissue infection. Moreover, *M. odoratimimus* was the causative agent accountable for an epidemic outbreak in a Turkish hospital

in 2010. Although the incidence is low the clinical relevance increases due to multiple antibiotic resistances of these opportunistic pathogens. However, almost nothing is known about virulence or host adaptations to of *Myroides* spp. Here we report an uncommon isolate of the genus *Myroides* isolated from a Foley´s catheter biofilm of a 91 year old female patient. Treatment of the catheter associated urinary tract infection of this patient with various antibiotics failed. The isolate was continuously present in the biofilm at the catheter/ uroepithelial interface so that we were able to isolate the same species one year later from a different catheter of the patient.
The *Myroides* isolate produces urease, extracellular DNase and hemolysin. Moreover, it is able to adhere to bladder epithelial cells. SEM micrographs unraveled uncommon cell morphology with branching rods and cap-like structures on the tip of the cells. Single cells grow up to 60 μm in nutrient broth. Antibiotic resistances include those to kanamycin, ampicillin, cefixime, nitrofurantoine, tobramycin, ciprofloxacin, gentamicin, streptomycin, carbenicillin, chloramphenicol, rifampicin, erythromycin, neomycin, spectinomycin and cotrimoxazole, respectively. Polyphasic taxonomic analyses did not yield a clearly species affiliation. The genomes of the two isolates were sequenced. Additionally, we analyzed metabolome and proteome of the strains under urinary tract like conditions.

MMAV015

Role of high-level amplification of β-lactamase genes in carbapenem resistant *E. coli*

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We have previously described that gene duplication and amplification (GDA) of the β-lactamases bla_{TEM-1}, bla_{OXA-1} and $bla_{CTX-M-15}$ in the outbreak plasmid pUUH239.2 contributed to carbapenem resistance [1]. To further study this mechanism of β-lactam resistance we selected high-level amplifications of these β-lactamases and characterized their effect on carbapenem resistance.
Bacteria (*ΔompC/ΔompF* with pUUH239.2) were selected on plates supplemented with carbapenems (meropenem and ertapenem) and mutants were assayed for the frequency of GDA. We performed continuous selections with increasing concentrations of carbapenems in Mueller-Hinton broth for 60 generations to select for high-level resistance and GDAs. Copy-numbers of b-lactamase genes were determined using quantitative real-time PCR (qRT-PCR) and the segregational loss rate of amplifications was determined. Whole genomes of individual clones from the endpoint of the selection were sequenced. The fitness cost of high-level resistance was measured using the BioscreenC reader (Labsystems). The selection was continued at constant high concentrations of carbapenems to select for compensatory mutations and gain-of-function mutations in the β-lactamases that increased their catalytic activity towards carbapenems.
The amplification frequency was 90% among isolates with increased minimal inhibitory concentrations (MICs) of carbapenems. Copy-numbers of bla_{OXA-1} and $bla_{CTX-M-15}$ were increased up to 80 copies after meropenem selection while selection with ertapenem only increased gene copy numbers up to 25 copies. Interestingly this lower level of GDA was sufficient to confer similar fold-increases of ertapenem and meropenem MICs. Bla_{TEM-1} was not present in the amplification regions. The fitness generally decreased strongly with increasing levels of amplifications and low-level amplifications after selection with ertapenem seemed to be as costly as high-level amplification after selection with meropenem.
Underlying compensatory and resistance mutations contribute to the fitness cost.

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MMAV016

Discriminative detection of methicillin resistant *Staphylococcus aureus* carrying panton-valantine leukocidin gene in clinical isolates

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Question: The emergence of Methicillin Resistant *Staphylococcus aureus* (MRSA) as a major cause of infection has become a global concern owning to the difficulty to combat due to the emerging resistance against all classes of antibiotics and the presence of several virulence genes. Panton-Valentine leukocidin (PVL) is one of the most important virulence factors in *S. aureus* and causes white blood cell destruction, necrosis and accelerated apoptosis. PVL-MRSA associated infections are recently found to be increasing at an alarming rate. This study was conducted for identification of *S. aureus* as well as prevalence of PVL and other virulence factors in MRSA.
Methods: A newly described hexaplex PCR strategy was used for the rapid detection of MRSA and virulence factors. A total of 640 clinical isolates were collected and screened from different sources including blood, wound and other infections such as pus, urine, HVS and CVP. Out of 640, 263 were confirmed after biochemical screening as *Staphylococcus aureus*. It targets the *nuc* (Specific for *S. aureus*), *mec A* (methicillin resistance determinant), *fem A* and *fem B* (*S. aureus* specific factors essential for methicillin resistance), *Luk S/F PV* (encodes for PVL) and *spa* (encodes protein A).
Results: Among 263 *S. aureus* isolates, 128 (48.67%) were screened out to be MRSA. The screening of MRSA revealed that 61 (47.6%) were PVL positive cases. In PVL positive cses, 27 (44.26%) were from blood, 20 (32.8%) were from wound and 11 (18%) were from pus whereas 3 (4.9%) were from other sources. Among isolates from blood samples, 49.1%, from wound 58.82%, from pus 52.38% and from other sources only 2% showed PVL positive.
Conclusions: This study concludes that the highest percentage of PVL positive samples were isolated from wound i.e. 58.82%. Systematic surveillance studies may contribute in determining the prevalence and in preventing widespread dissemination of PVL-MRSA associated infections, facilitating antibiotic therapy design.

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MMAP001

Pituitary adenylate cyclase-activating polypeptide (PACAP) treatment ameliorates acute small intestinal inflammation in mice

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Background: Pituitary adenylate cyclase-activating polypeptide (PACAP) plays a crucial role in immunity and inflammation. For the first time, we investigated the potential anti-inflammatory and immuno-modulatory properties of PACAP in a murine model of hyper-acute Th1-type ileitis following peroral infection with *Toxoplasma (T.) gondii* thereby mimicking immunopathological key features of acute Crohn's Disease.
Methodology/Principal Findings: After intraperitoneal PACAP administration (1.5 mg / kg body weight / day, once daily, for six days), mice were protected from ileitis development whereas all placebo controls suffered from small intestinal necrosis and were prone to death by day 7 following *T. gondii* infection. PACAP treated animals displayed significantly increased numbers of regulatory T cells in the ileum mucosa compared to placebo controls. In contrast, mucosal T lymphocyte, neutrophilic granulocyte as well as macrophage and dendritic cell numbers were reduced in PACAP treated mice. In addition, levels of the anti-inflammatory cytokine IL-4 (in MLNs, liver) and IL-10 (in spleen, serum) were increased whereas pro-inflammatory cytokine expression was found to be significantly lower in the ileum (IFN-gamma, MCP-1), draining mesenteric lymph nodes (IFN-gamma, nitric oxide), spleen (TNF-alpha, nitric oxide), and liver (IFN-gamma, MCP-1, IL-6, TNF-alpha) following PACAP treatment as compared to placebo. Furthermore, treated animals displayed more than five times higher concentrations of potentially anti-inflammatory lactobacilli in their ileum lumen, as compared to non-treated controls. Finally, PACAP treatment preserved intestinal barrier functions as

indicated by lower translocation rates of live bacteria into spleen, liver, kidney, and blood as compared to placebo controls.
Conclusion/Significance: PACAP treatment ameliorates acute small intestinal inflammation by down-regulating Th1-type immune responses, reducing oxidative stress, and up-regulating extraintestinal IL-4 and IL-10 expression, and reduces bacterial translocation by maintaining gut barrier function. These findings provide potential prophylaxis and treatment options for patients suffering from inflammatory bowel diseases.

MMAP002
Interaction of *Corynebacterium ulcerans* with host cells

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Corynebacterium ulcerans is a pathogenic species of the genus *Corynebacterium* and closely related to the human pathogen *Corynebacterium diphtheriae* and the veterinary pathogen *Corynebacterium pseudotuberculosis*. *C. ulcerans* is mainly associated with mastitis in cattle, non-human primates and other animals and is also known as commensal in various domestic and wild animals. However, during the last decade, human infections associated with *C. ulcerans* appear to be increasing in various countries and can most often be ascribed to zoonotic transmission. Besides respiratory diphtheria-like illness, *C. ulcerans* can also cause extrapharyngeal infections in humans, including severe pulmonary infections.
To date, only little is known about mechanisms that this emerging pathogen uses for host colonization. Here, adhesion to and invasion in eukaryotic cells by *C. ulcerans* wild type strains and corresponding mutants in putative virulence factors were examined. Furthermore, intracellular survival and replication assays in macrophages were carried out. These cell-based infection models revealed that *C. ulcerans* adheres to and enters epithelial cells in strain-specific amounts and that this bacterium can survive and replicate inside macrophages. As another infection model to evaluate inter-strain variation in pathogenicity, larvae of the greater wax moth *Galleria mellonella* have been infected with *C. ulcerans*. All *C. ulcerans* strains exhibited high virulence towards the larvae resulting in melanization and death.
Although *C. ulcerans* is closely related to *C. diphtheriae* and can in principal harbor the diphtheria toxin as well, the strains examined here lack the genomic information for this toxin. Interestingly, *C. ulcerans* showed higher virulence to eukaryotic cells compared to *C. diphtheriae*, suggesting other factors besides the diphtheria toxin which are crucial for *C. ulcerans* virulence. This and the fact that *C. ulcerans* is able to survive and replicate within phagocytic cells, makes this organisms of greater interest with respect to systemic infections.

MMAP003
The *Pseudomonas aeruginosa* pangenome – impact of genomic diversity on bacterial pathogenicity and host response in airway infections

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Introduction: The multidrug resistant *Pseudomonas aeruginosa* is a metabolically versatile opportunistic pathogen. It has become the worldwide most common gram-negative pathogen for community-acquired and nosocomial pneumonia including ventilator-associated pneumonia (VAP) in intensive care units. Infections of the respiratory tract with *P. aeruginosa* moreover contribute substantially to morbidity and mortality in individuals with chronic obstructive pulmonary disease (COPD). The prevalence of chronic airway infections with *P. aeruginosa* in COPD is about 600,000 cases in Germany thus making it to one of the most frequent severe infections in this country.
Objectives: This research project has the aim of resolving the pangenome, transcriptome and proteome of *P. aeruginosa* and identifying the genomic differences between strains with high, low or even negligible pathogenic potential for mammals.
Results: Sequence analysis was performed on VAP and COPD isolates, which represent the most common clones of the *P. aeruginosa* population and reference environmental strains lacking isolation from the clinics.
Pathogenicity of the completely sequenced strains was compared in *in vitro* and *in vivo* airway infection models. The pathogenic screening of the 20 strains in an acute murine infection model showed significant differences in the course of infection. Mice infected with the most attenuated strain (B420) showed no symptoms of infection. In contrast, a set of 5 strains was highly virulent causing a severe infection whereby mice deceased within 72 h p.i. The most virulent strain (F469) was even able to replicate intracellularly in the lung tissue. Thus, *P. aeruginosa* strains can be clearly distinguished from one another in their pathogenic potential.
In silico analysis of the 20 sequenced genomes revealed a distinct SNP pattern compared to the reference genome PAO1 for strain B420 and F469. B420 is furthermore missing a set of genes compared to PAO1, which are relevant for virulence.
Outlook: In the next step the impact of bacterial genomic diversity on the course of airway infections will be assessed in an acute murine airway infection model. The infectious process will be assessed by cytokine level, bacterial colonization and by the quantitative RNA-Seq, protein and lipid profiling of murine lungs.

MMAP004
Development of real time PCR assays for rapid detection of resistance against ciprofloxacin in *Bacillus anthracis*

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The zoonotic infectious disease anthrax is caused by *Bacillus anthracis*. Common therapy against anthrax typically comprises primarily the antibiotic ciprofloxacin. Resistances against the gyrase-inhibitor ciprofloxacin occur in this Gram-positive bacterium and are described in the literature. To test for resistances in a *B. anthracis* strain from a patient with an ongoing anthrax infection resistance-assays such as the antimicrobial diffusion gradient or micro-dilution methods are applied. But this kind of diagnosis requires up to two days. Therefore, we developed a more rapid method involving real time PCR assays to identify mutations leading to resistance against ciprofloxacin in *B. anthracis*.
Because of dual-use issues associated with *B. anthracis* being a BSL-3 organism we created ciprofloxacin resistant strains employing a less pathogenic very close relative. DNA-sequences especially of the fluoroquinolone resistance determining (gene) regions (QRDR) are highly conserved between *B. cereus* and *B. anthracis*. *B. cereus* ATCC10987 which is a BSL2-pathogen was made fluoroquinolone-resistant via natural selection of resistant strains by cultivating the bacteria on agar plates containing increasing ciprofloxacin concentrations. We identified single nucleotide polymorphisms (SNPs) in the QRDR of gyrase and type IV topoisomerase genes. These QRDR comprise the genes *gyrA*, *gyrB*, *parC* and *parE* which represent the targets of ciprofloxacin. Mutations in these genes are known to cause resistance against ciprofloxacin.
The diagnostic PCR-assays are based on one of two real time methods, (i) TaqMan® or (ii) Melt Analysis of Mismatch Amplification Mutation Assays (MeltMAMA). We are currently comparing both methods using wild-type controls and the ciprofloxacin resistant *B. cereus* mutants as proxies for *B. anthracis*. Next, spiked human samples will be used to determine the sensitivities and specificities of the developed rapid PCR-assays.

MMAP005
Identification and characterization of the MurT/GatD enzyme-complex catalyzing lipid II amidation in *S. aureus* and the impact of lipid II amidation on defensin and glycopeptide susceptibility

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The peptidoglycan of *Staphylococcus aureus* is characterized by a high degree of crosslinking and almost completely lacks free carboxyl groups, since the α-carboxyl group of D-glutamic acid at position 2 of the stem

peptide is amidated, resulting in the formation of D-iso-glutamine. Amidation of peptidoglycan has been proposed to play a decisive role in polymerization of cell wall building blocks, correlating with the efficient crosslinking of neighboring peptidoglycan stem peptides. Antisense-mediated depletion in expression of GatD and MurT led to restored susceptibility to diverse β-lactam antibiotics [1] and the defensin plectasin in MRSA strain COL. Moreover, determination of the binding parameters to lipid II and amidated lipid II, using quartz crystal microbalance (QCM) biosensor technique revealed a dramatically reduced binding affinity of plectasin to the amidated cell wall building block. Reconstitution of the MurT/GatD-catalyzed reaction *in vitro* confirmed that the cell wall building block lipid II and/or lipid II-Gly_5 is the preferred substrate of the bi-enzyme complex [2]. BLAST analysis of MurT further revealed the presence of a C-terminal domain of unkown function (DUF*1727*) lacking in other Mur-ligases involved in peptidoglycan synthesis.
For further characterization of this domain and its possible molecular function, we constructed various mutants of MurT and tested them *in vitro* for activity and interaction with GatD.

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MMAP006

In vitro reconstitution of capsule biosynthesis reactions and regulation by the CapAB kinase complex in *Staphylococcus aureus*

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Staphylococcus aureus is a major cause of nosocomial infections responsible for a diverse spectrum of human diseases, like wound and several other invasive infections. Commonly, most microorganisms, that cause invasive diseases, produce extracellular capsular polysaccharides that enhance their virulence and protect the pathogen from phagocytosis and opsonization by antibodies [1].Beside the capsule biosynthesis enzymes, *S. aureus* genome analysis reveals two polypeptides, CapA and CapB, harboring a putative tyrosine kinase activity [2] and seem to regulate the capsule biosynthesis.
Despite of the importance of the capsule for pathogenicity, capsule biosynthesis is not fully understood on the molecular level. Especially the membrane bound steps of the biosynthesis pathway and the regulation by the tyrosine kinase CapB remain largely unclear.
Reconstitution of capsule biosynthesis reactions *in vitro*, resulting in the formation of lipid-bound precursors lipid I_{cap} and/or lipid II_{cap}, is only possible in the presence of the tyrosine kinase complex CapAB.
In vitro phosphorylation assays further identified the epimerase CapE as a novel substrate of the kinase CapB, which is most likely controlling the CapE-catalyzed synthesis of the soluble capsule precursor UDP-L-FucNAc.

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MMAP007

The meaning of pili in *Enterococcus faecalis*

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Microbial biofilm formation is a wide-spread life-style of bacteria in nature. Especially occurrence in human infections and the difficult treatment of such biofilm based diseases represents a severe problem (1). In the multi-stage biofilm formation process, we are particularly interested in the first step, which is in addition to other factors mediated by pili. In *Enterococcus faecalis* the predominant pilus is encoded by the *ebp*-locus (2, 3). But in a little percentage of *E. fc* isolates a second gene cluster, called *bee*-locus (*b*iofilm *e*nhancer in *e*nterococci), has been identified (4, 5). Sequence analysis of the clinical isolate *E. faecalis* 1.10 revealed a new arrangement of the *ebp*-locus, resulting in the loss of the pilus. At the same time this isolate harbors the second pilus locus on the conjugative plasmid pBEE99, mediating still strong biofilm formation strength. Comparing the isolate with an isogenic biofilm defective mutant and employing several different approaches including sequencing, western blotting, mass spectrometry and phenotypic assays, we were able to show the necessity of minimum one pilus species at the surface of the Gram positive cell to start biofilm formation by initial adherence. Insertion of IS elements into the *ebp* gene cluster caused the loss of the Ebp pilus. This defect can be balanced by expression of an additional pilus-locus, called *bee*. A double negative pilus mutant showed weak biofilm formation, indicating the importance of pilus-directed attachment to surfaces and subsequent biofilm formation. Additionally we have shown the integration activity of sortase 1 concerning the pilus subunit Bee2 of intact Bee-pilus.

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MMAP008

Targeting intracellular *Legionella* via anti-virulence compounds

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Legionella pneumophila is a Gram-negative bacterium which is capable of hijacking and replicating within their aspiring predators, phagocytic amoeba (*1*). The molecular mechanisms responsible for amoeba-resistance also allow replication within human macrophages, rendering *L. pneumophila* an accidental, but deadly, human pathogen. The intracellular growth phase of *L. pneumophila* provides a challenge for traditional antibiotic development, and new compounds need to take the effects of the host-pathogen environment into account.
We report here on the development of a novel assay for the intracellular growth of *L. pneumophila* within a natural environmental host, *Acanthamoeba castellanii*. Characterisation of this fluorescence-based assay revealed the ability to distinguish altered intracellular replication rates caused by gene deletion or drug treatment, and allowed the quantification of the degree of protection afforded to intracellular bacteria. To examine the role of alterations to the host cell we tested several putative "anti-virulence" compounds; including inhibitors of cytoskeletal formation, vesicle scission, and Ras kinase localisation. Our results indicate a hitherto unrealized antibiotic property of the Ras depalmitoylation inhibitor, Palmostatin M. Further characterisation indicated that this compound, but not the closely related Palmostatin B, caused specific growth inhibition in the *Legionella* and *Mycobacterium* families, raising the interesting possibility of a common target protein.

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MMAP009

Role of phosphoinositide lipids for pathogen vacuole formation of *Legionella longbeachae*

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The opportunistic pathogenic bacteria of the genus *Legionella* manipulate their host cells (amoeba and macrophages) in order to live and thrive inside a unique compartment, called the "*Legionella*-containing vacuole" (LCV). More than 275 "effector proteins" that are translocated into the host cell via the bacterial Icm/Dot type IV secretion system (T4SS) assist in establishing this replicative niche. Several of these effectors anchor to phosphoinositide (PI) lipids on the cytosolic face of LCVs, where they interfere with host cell vesicle trafficking and signal transduction pathways [*1*].
L. pneumophila and *L. longbeachae* both cause the severe pneumonia Legionnaires' disease. Remarkably, important differences in the genetic repertoire of the two species were identified and suggested different strategies for intracellular replication and niche adaptation, in particular as *L. longbeachae* possesses a unique arsenal of putative Icm/Dot T4SS effectors, some of them eukaryotic-like proteins.
Currently, we investigate the role of several *L. longbeachae* effectors in establishing the replicative vacuole by employing and subverting host cell PIs. Recent studies identified the *L. longbeachae* SidC homologue as an Icm/Dot T4SS substrate localizing to the LCV membrane. *L. longbeachae* SidC (but not the *L. pneumophila* homologue) bound to PI(4)*P* in pulldown experiments using different PI lipids coupled to agarose beads. Protein-lipid overlay assays revealed that *L. longbeachae* SidC specifically binds to PI(4)*P* and identified the 20 kDa PI-binding domain. Current efforts aim at a detailed functional characterization of *L. longbeachae* PI-interacting effector proteins and an analysis of purified intact *L. longbeachae* LCVs.

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MMAP010

Domain structure and interaction of the Multiple Peptide Resistance Factor (MprF)

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The lysinylation of negatively charged phosphatidylglycerol by MprF proteins reduces the affinity of cationic antimicrobial peptides (CAMPs) and the antibiotic daptomycin for bacterial cytoplasmic membranes and confers resistance to CAMPs in several Gram-positive bacterial pathogens. MprF of *Staphylococcus aureus* consists of separable domains for lysyl-phosphatidylglycerol (Lys-PG) production and Lys-PG flipping (*Ernst et al., PLoS Pathogens 2009*). However, it has remained unclear if the two functional parts are overlapping and which of the various trans-membrane domains (TMDs) are required for flippase activity.
By analyzing the activity of various mutated flippase variants, we found that the core flippase encompasses the N-terminal 8 TMDs of MprF, prompting us to investigate the interaction of the flippase with the synthase in a bacterial two hybrid assay. The employment of various combinations of MprF subdomains demonstrated that the synthase interacts with the flippase and revealed a specific intra-domain interaction pattern which appears to lead to oligomerization of MprF.
These findings have important implications for studying the role of daptomycin resistance-associated point mutations in MprF and for developing MprF inhibitors.

MMAP011

Extended-spectrum beta lactamase producing bacteria in livestock husbandry in manure and output samples of biogas plants

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Extended-spectrum beta lactamase producing *Enterobacteriaceae* (ESBLs) are an increasing problem of human infections. Intensive application of antibiotics in livestock husbandry increases the occurrence of antibiotic-resistant bacteria which can be released by manure into soil, ground and surface waters and can thereby enter the human food chain. Alternatively to a direct application of manure on fields, manure is used as input material of biogas plants to produce biofuels. The transmission of antibiotic-resistant bacteria through biogas plants has not been investigated so far. In this project, ESBLs were detected in manure of livestock's husbandry and the output of biogas plants to investigate if biogas plants can prevent or reduce the transmission of ESBLs to soils, ground and surface waters. Therefore we determined ESBLs in input and output samples of eight biogas plants using cultivation dependent and molecular cultivation independent methods.
ESBLs were cultured on commercial ESBL CHROMAgar (MAST Diagnostica, Reinfelde, Germany) including a specific pre-enrichment. Colonies grown on ChromAgar were screened by Multiplex-PCR for the abundance of the three prevalent ESBL genes (CTX-M, TEM and SHV). A more detailed characterization of the ESBLs included phylogenetic identification by 16S rRNA gene sequencing, ESBL type determination by sequencing of the ESBL genes, and determination of the resistance spectrum with special designed veterinary antibiotic microtiter plates. In parallel ESBL genes were directly determined in DNA extracts of manure and biogas plant output samples.
So far CTX-M and TEM ESBLs were detected in manure but not after the transfer through biogas plants with one exception after specific pre-enrichment increasing the detection efficiency. SHV genes were not detected at all. Isolated ESBLs were affiliated to the genera *Escherichia*, *Achromobacter*, *Citrobacter*, *Serratia* and *Shigella*. Most of the ESBLs showed also a high resistance level to most of the testes antibiotics. Our results indicate a high abundance of multi-resistant ESBLs in manure for livestock husbandry and indicate a reduction of ESBLs after the transfer through biogas plants.

MMAP012

The role of the pneumococcal methionine sulfoxide reductases in the resistance against intra- and extracellular oxidative stress and maintenance of virulence

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The respiratory pathogen *Streptococcus pneumonia*e (the pneumococcus) is an important pathogen colonizing as a commensal the human upper respiratory tract but also causing severe and life-threatening invasive diseases. Its location in the human respiratory tract forces the bacterial cell to develop mechanisms to resist the host defenses like the oxidative burst produced by the innate immune system components such as reactive oxygen species (ROS). ROS originating from monoelectronic reduction of O_2 and ROS radical species such as hydrogen peroxide, hydroxyl radical ($HO^{\cdot}$), and superoxide anions ($O_2^{\cdot-}$) are reported to damage several bacterial central constituents including proteins by spontaneous oxidation of exposed highly susceptible methionine (Met) residues to methionine sulfoxide (MetO). Consequently the induced conformational change leads to a loss of protein function. This reaction is reversible and damaged proteins can be repaired by methionine sulfoxide reductases (Msr) converting the oxidized methionine sulfoxide back to methionine. MsrA and MsrB occur typically as separate enzymes. In some bacteria like *S. pneumoniae*, *Neisseria gonorrhoeae,* and *Haemophilus influenzae* both proteins, MsrA and MsrB, are translationally fused to a single protein. We have identified a two-operon system responsible for the extracellular oxidative stress resistance in *S. pneumoniae*. This system is composed of two integral membrane proteins (CcdA1 and CcdA2), two thioredoxin lipoproteins (Etrx1 and Etrx2) and a single methionine sulfoxide reductase (*Sp*MsrAB2), which shares high homologies in the MsrA and MsrB domains to the cytoplasmic *Sp*MsrAB1. The impact of both MsrAB proteins on oxidative stress resistance, phagocytosis and virulence was assessed using mutants deficient in one or both *Sp*MsrAB proteins. The deficiency of both SpMsrAB1 and 2 enhanced oxidative damage of proteins and accelerated pneumococcal killing by H_2O_2. In addition, the lack of one MsrAB proteins attenuated significantly pneumococcal virulence in the acute pneumonia model and loss-of-function of both *Sp*MsrAB proteins had an additive effect. In conclusion, this oxidative stress resistance system is a critical component of the pneumococcal reactive oxygen species defense system being essential for repairing intra- and extracellular proteins.

MMAP013

Identification of medically important fungi using MALDI-ToF

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Fast identification of medically important moulds and dermatophytes is not well established yet. There are some selective agars and some first evaluations of PCR-based kits. Identification by using selective agars needs a lot of time, because most dermatophytes are growing slowly and then it needs further time until exact microscopical identification is possible. Identification with PCR-based kits needs less time, but they are not well established in diagnostic laboratories yet. Usually, they do not detect all species and are connected to high costs. A fast identification of pathogenic organisms is very important to prevent dissemination and to apply a suitable therapy, that occurs only with exact differentiation of these organisms. According to fungi, the source of infection has to be found, e.g. whether infection comes from contaminated rooms or from baths, so the patient is not longer exposed to the infectious material and therapy takes specific and fast effect.
We established identification of fungi by using Matrix-Assisted Laser Desorption Ionization - Time of Flight Mass Spectrometry as established by Alshawa et al. 2012 and Theel et al. 2011. The advantages over identification with PCR-kits and gene sequencing are faster identification, lower costs and multiple application without using big volumes of cancerogen chemicals. Bacteria can be identified as well as fungi. Moulds and dermatophytes were grown at 30 degree celsius for 3 and 7 days on

SAB-GC and Potatoe-Dextrose-Agar. Then we did Pistil-Ethanol-Formic Acid extraction as described in the manual of Bruker Daltonik GmbH. After that we imported the measured spectra in our Bruker Database. With a dataset of *Aspergillus versicolor*, as example for fungi, we will show that growing conditions belonging to nutrition influence mass peaks and also the quality of produced data. Statistics of identification will be shown as well as indexes of correlation belonging to consistency of data, e.g. danger of confusion between two strains.
We also will show the dependance of growth time on MALDI-ToF result for some species.

Kinda Alshawa, Jean-Luc Beretti, Claire Lacroix, Martine Feuilhade, Brunhilde Dauphin, Gilles Quesne, Noura Hassouni, Xavier Nassif and Marie-Elisabeth Bougnoux. J.CLin.Microbiol. 2012, 50(7):2277. DOI 10.1128/JCM.06634-11.
Elitza S. Theel, Leslie Hall, Jayawant Mandrekar and Nancy L. Wengenack. J.Clin.Microbiol. 2011, 49(12):4067. DOI 10.1128/JCM.01280-11.

MMAP014

The periplasmic domain of Intimin mediates dimerisation and binding to peptidoglycan

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Intimin and Invasin are well-characterised virulence factors of enterophathogenic *Escherichia coli* and yersiniae, respectively. These outer membrane proteins belong to a family of proteins whose extracellular domain is secreted through the outer membrane by a novel autotransport mechanism, termed type Ve secretion [1]. Compared to classical (type Va) autotransporters, Intimin and Invasin have an inverted topology, with the C-terminal passenger being exported through an N-terminal β-barrel pore [2]. In addition, these proteins have a small N-terminal periplasmic domain. The periplasmic domain of Intimin contains a lysine motif (LysM) region found in many peptidoglycan-binding proteins, but this motif is lacking in Invasin. We show that the periplasmic domain of Intimin, but not the smaller domain of Invasin, binds to peptidoglycan sacculi. The binding is pH-dependent and occurs only under acidic conditions (pH ≤ 6.0). Furthermore, the Intimin periplasmic domain mediates dimerisation. In contrast to peptidoglycan binding, dimer formation by the Intimin periplasmic domain is independent of pH. The Invasin periplasmic domain does not promote dimerisation under any of the tested conditions. We are currently performing experiments to determine the region(s) in the periplasmic domain responsible for peptidoglycan binding and dimerisation, testing the relevance for dimerisation and peptidoglycan binding for host cell adherence and pedestal formation.

1. Leo JC, Grin I, Linke D, *Philos Trans R Soc Lond B Biol Sci*., **2012**, *367*, 1088-1101
2. Oberhettinger P, Schütz M, Leo JC, Heinz N, Berger J, Autenrieth IB, Linke D, *PLoS ONE* **2012**, *7*, e47069

MMAP015

Humanized anti-staphylococcal anti-bodies - a novel tool against MRSA

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Introduction: The gram-positive bacterium *Staphylococcus aureus* is the major cause of nosocomial infections. In particular, diseases caused by methicillin-resistant *S. aureus* (MRSA) are associated with higher morbidity, mortality and medical costs due to showing multiple resistances to several classes of established antibiotics. Furthermore, this microorganism is able to develop resistance mechanisms against new antibiotics rapidly. Therefore, immunological strategies based on therapeutic antibodies have the potential to become a powerful tool for the treatment of MRSA. The immunodominant staphylococcal antigen A (IsaA) has been identified as a potential target for immunotherapy due to its expression by the majority of relevant clinical strains *in vivo*, and its surface accessibility for antibodies. First preclinical experiments in different mouse infection models revealed protective properties of the mouse anti-IsaA antibody (UK-66). In addition, *in vitro* phagocytosis assays showed enhancement of antibody dependent killing of *S. aureus* in the presence of UK66. Therefore, the mouse monoclonal UK-66 antibody was selected for humanization as a prerequisite for clinical studies in humans.
Results: The antibodies of the hybridoma clone UK-66 were the basis for the identification of the antibody binding domain against IsaA. The coding sequence was used to construct recombinant IsaA binding mouse scFv fragments and to humanize the murine antigen binding domain. After the characterization of the mouse and humanized scFv fragments in various binding studies (Western Blot, ELISA and FACS studies), the complete UK-66 antibody was constructed and tested. The affinity of the humanized UK-66 is comparable with the affinity of the mouse UK-66. The functional characterization of the complete UK-66 antibody was studied in opsono-phagocytose assays with purified human granulocytes and human whole blood. The assays revealed an antibody dependent killing of *S. aureus* Newman.
Conclusion: The results present the successfully humanization of the mouse UK-66 antigen binding domain. Based on the results the humanized UK-66 has the potential to close the gap for an efficient treatment of MRSA.

MMAP016

Antibiotic translocation through bacterial porins - insights from electrophysiology

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The outer membrane of Gram-negative bacteria contains a number of channel-forming hydrophilic proteins called porins. Porins allow diffusion of low-molecular weight solutes across the outer membrane. We use electrophysiology to study the molecular aspects of antibiotic translocation across porins with planar lipid bilayers. In this technique, purified porins are individually inserted into the bilayer. Permeation of molecules through the channel causes measurable fluctuations in the ion current. Thereby we are able to monitor the molecular interactions with the channel wall.
We have been able to characterize facilitated translocation of antibiotics, including beta-lactams and fluoroquinolones through various outer membrane channels of *Providencia stuartii*, *Burkholderia pseudomallei*, and *Escherichia coli*. Titration with effective antibiotics revealed concentration-dependent blockages of the ion flow suggesting interaction with the channel. Noise analysis of ion currents through porin in the presence of antibiotics enabled us to determine binding kinetics and transport parameters of these molecules at single-molecule level. The impact of temperature on the antibiotic passage through the porins has been investigated in the bilayer as well. The results obtained from bilayer measurements correlated well with i*n vitro* activity of antibiotics determined by MIC assays. Experimental results were compared with molecular dynamics simulations which provide the energy barriers along the diffusion pathway and an atomic description of the antibiotic translocation through porins. Our study of antibiotic translocation at single-molecule level gives new insights to design novel drugs with optimal penetration into bacterial cells.

MMAP017

Using transcriptional network analysis to identify an essential regulator for the mode of action of biofilm inhibitor carolacton

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The low susceptibility of biofilms to conventional antibacterial drugs emphasizes the urgent need for novel strategies and substances to erase pathogenic bacteria in biofilms, in particular in the context of rising antibiotic resistance. Myxobacterial substance carolacton (Kunze *et al*. (2010)) was found to be highly active against biofilms of Streptococci.
A comparative time-resolved transcriptome profiling of treated and untreated *S. mutans* was performed to decipher the mode of action of the substance and revealed the disturbance of cell wall metabolism, cell division, pyrimidine metabolism and two component signalling (TCS). The eukaryotic-type serine/threonine protein kinase PknB, displaying an alternative signalling system to TCS in prokaryotes, confers susceptibility to carolacton treatment (Reck *et al*. (2011)).

Here we use a systems biology approach to identify how carolacton disturbs cell division and cell wall metabolism in *S. mutans*. For the first time a transcriptional regulatory network was constructed in this organism using reverse engineering and trend correlation (TC) method. Five transcriptional key regulators (FabT, Rgg, CpsY, GlnR, SpxA) were predicted to be essential for the immediate cellular response (5-20 minutes) to carolacton treatment. Gene deletion mutants of these regulators were tested for their susceptibility to carolacton treatment. A deletion mutant of the CpsY regulator was found to be insensitive to carolacton treatment. CpsY was identified to be essential for the cell wall adaptations important for the intracellular survival of *S. iniae* in Neutrophils and for confering resistance to lysozyme treatment. Moreover deletion of CpsY significantly changes muropeptide composition, cell surface charge and peptidoglycan acetylation in this organism (Allen *et al*. (2012)). However the role of CpsY was never systematically addressed in *S. mutans*. To this end we use transcriptome and proteome profiling of carolacton treated and untreated *cpsY* gene deletion mutants to decipher how CpsY regulates cell wall adaptation in *S. mutans* essential for the survival of this oral pathogen and for the mode of action of carolacton.

MMAP018
Inactivation of orthopoxviruses via contact to metallic copper surfaces

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Orthopoxviruses (OPVs) are a group of notorious pathogens including some zoonotic members affecting livestock and humans. The most infamous member is variola virus, the causative agent of smallpox. Because of its potential of being misused as a bioterroristic agent causing major public health problems, variola virus is classified as a category A agent by the CDC.
The antibacterial and antifungal properties of metallic copper (Cu) surfaces are well known. Dry Cu-surfaces demonstrated their efficacy in inactivating a wide variety of microorganisms both under laboratory conditions and in hospital trials. Most microbes exposed to Cu-surfaces are rapidly inactivated within minutes by a quick and sharp Cu-overload shock in a process termed "contact-killing". However, antiviral properties of Cu surfaces are less well understood.
In this study the efficacy of metallic Cu against the orthopoxvirus species vaccinia, cowpox (both BLS2) and monkeypox virus (BSL3) was determined. For this, the infectivity of three reference strains which had been pre-exposed to Cu surfaces was determined on African Green Monkey kidney cells. Preparations which had been pre-exposed to stainless steel surfaces served as controls. Infected cell cultures were fixed, stained with crystal violet and virus titers were determined by plaque counting.
The killing-kinetics demonstrated efficient and rapid inactivation of OPVs upon contact to dry Cu-surfaces. In contrast, stainless steel control-surfaces did not exhibit any antiviral properties. This is the first description of the antiviral activity of Cu against DNA-viruses, in particular human- and animal-pathogenic orthopoxviruses.

MMAP019
Proteomic characterization of different *Legionella pneumophila* life stages

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The Gram-negative bacterium *Legionella pneumophila* is the causative agent of Legionnaires' disease, a severe human pneumonia. In the natural environment, *L. pneumophila* colonizes biofilms and parasitizes amoebae. The intracellular life cycle of *L. pneumophila* is divided into two distinct stages: the replicative phase, where the bacteria multiply until the nutrients cease, and the transmissive phase, where the bacteria render virulent and invasive. Similar stages are also defined during growth in broth medium. Upon prolonged periods of stress, such as nutrient deprivation and temperature change, *L. pneumophila* enters into the viable but not culturable (VBNC) state where the bacteria show a low level of metabolic activity and do not grow on standard media. VBNC state *L. pneumophila* may resuscitate and thereby regain culturability and virulence after passage through amoebae. In consequence, VBNC state *L. pneumophila* has to be considered as a public health hazard. We here aimed to characterize the different *L. pneumophila* life stages by means of a systematic proteomic comparison of the replicative phase, the transmissive phase, and the stress-induced VBNC state. To induce VBNC cell formation in *L. pneumophila*, different stress conditions, like cold and heat stress, nutrient limitation, and several chemical agents, were tested. Culturability in microcosms at 4°C and 21°C did not decrease for at least 400 days. Contrarily at 42°C, the number of CFU decreased from $2x10^8$/ml to zero within 68 days. About 40% of the bacteria still remained intact according to live/dead staining and microscopic analysis regardless of the dramatic decrease in CFU counts. Proteomic comparison of broth-grown phases and stress-induced VBNC *L. pneumophila* is currently in process and will contribute to a deeper understanding of bacterial modification processes in the distinct life stages and may identify important marker proteins for improved pathogen detection.

MMAP020
Staphylococcus aureus Panton-Valentine-Leucocidin causes neutrophil destruction resulting in activation of platelets which can be inhibited by plasma antibodies against leucocidins

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Panton-Valentine Leucocidin (PVL) is a bicomponent pore-forming exotoxin produced by S*taphylococcus aureus*. PVL expressing strains are highly associated with severe infections, like necrotizing pneumonia or severe osteomyelitis with thrombosis in children. This study was designed to investigate the interaction of PVL with neutrophils and platelets to explain the abnormal pathology of thrombosis in young patients.
We treated freshly isolated human platelets with PVL in presence or absence of human neutrophils and measured the activation status of the platelets using flow cytometry. The cytotoxic effect of PVL on neutrophils was evaluated by propidium iodide uptake. Platelet poor plasma of different blood donors were added in the experiments with the neutrophils alone and in the experiments with platelets and neutrophils. The antibody titer against leucocidins of the blood donors was determined by ELISA.
We found that PVL induced rapid cell death (within 20 min) in neutrophils, but did not have an impact on platelets alone. In contrast, platelets were activated after PVL addition in presence of neutrophils. This can be explained by the release of myeloperoxidases, human neutrophil peptides and reactive oxygen from PVL-damaged neutrophils, which are known as strong activators of platelets. The cytotoxic effect of PVL on neutrophils as well as the resulting activating effect on platelets could be blocked by the addition of human plasma of some blood donors. The inhibiting effect of plasma on neutrophils destruction by PVL correlated with the amount of antibodies against PVL in the plasma of the different donors.
Taken together, we could demonstrate that PVL acts on platelets indirectly via neutrophil destruction as it was already shown for epithelial cells (Niemann *et al*., 2012). The protection of antibodies in the plasma could explain why young patients are more likely to develop a PVL-*S. aureus* associated thrombosis than older patients, who are more likely to have developed a protective antibody titer against PVL.

Niemann S., *et al*. J Infect Dis. 2012 Oct 1; 206(7):1138-48.

MMAP021
Structural analysis of the type three secretion system export apparatus

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Type III secretion systems are widely spread virulence factors in Gram-negative pathogens that are used to deliver proteins from the bacterial cytoplasm into the host cell. Type III secretion systems are made up of a well-studied needle complex, which connects the bacterial and host cytoplasm, and a far less well-defined export apparatus located at the inner membrane center of the needle complex. The export apparatus plays an important role in substrate recognition, specificity switching and translocation over the inner membrane. We use *Salmonella enterica* serovar Typhimurium as a model organism to determine the makeup of the export

apparatus. The substituted cysteine accessibility method is used to assess the topology of its components while a mass-spectrometry based approach is employed to assess its stoichiometry. The knowledge of the structural properties of the export apparatus components provides a fundament for the further functional characterization of the apparatus and hence the type III secretion.

MMAP022

Surveillance of carbapenemase producing *Enterobacteriaceae* in the Netherlands in 2012

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Introduction: The spread of carbapenemase producing members of the *Enterobacteriaceae* family (CPE) is a worldwide emerging public health threat. In 2009, the first outbreak of *Klebsiella pneumoniae* isolates harboring the *Klebsiella pneumoniae* carbapenemase (KPC) gene occurred in theNetherlands. This triggered the need for a nationwide surveillance ofCPE to monitor this threat. In 2011, this task was assigned to the National Institute of Public Health and the Environment (RIVM). Medical microbiological laboratories in the Netherlands were requested to submit colonies suspected of carbapenemase activity to the RIVM for confirmation, free of charge. The criterium for submission was a meropenem MIC of > 0.25.
Methods: Confirmation is done both geno- and phenotypically. Genotypic confirmation consists of PCR's for eight known carbapenemase genes: KPC, NDM, OXA48, VIM, IMP, GIM,SIM and SPM. Phenotypic confirmation is done in three ways: first an Etest to confirm decreased susceptibility for meropenem. Secondly, disk diffusion synergy tests with combination disks (meropenem, meropenem+EDTA, meropenem+cloxacillin and meropenem+boronic acid) to identify different resistance mechanisms. Finally, carbapenemase activity is tested using the Modified Hodge Test.
Results: In 2012, over 250 CPE suspected isolates were submitted to the RIVM, mainly *Klebsiella* (38%) and *Enterobacter* (34%), but also *E. coli* and *Proteus* (both 10%). In 27% of these, a carbapenemase gene was found. Most commonly found gene was OXA48 (15%), followed by NDM and KPC (6% and 4%) and finallyIMP and VIM (2% and 1%). Until now, GIM,SIM and SPM have not been found in the surveillance yet.
Conclusion: Because of the voluntary basis of the surveillance and isolate submission, these statistics are biased and can only be regarded as an indication for the true prevalence of CPE in theNetherlands. The indication these findings provide is that CPE does not seem to occur at a high frequency in the Netherlands. However, experiences from other countries and previous outbreaks indicate that they pose a significant public health threat and should therefore be closely monitored.

MMAP023

Comparing the presence of siderophore related genes in *Salmonella* subspecies I - IV and development of a diagnostic multiplex PCR method for identification of Reptile-Exotic-Pet-Associated-Salmo-nellosis (REPAS)

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Reptile-Exotic-Pet-Associated-Salmonellosis (REPAS) in humans has become a growing problem in Europe. Reptiles are frequently asymptomatic carriers of *Salmonella* and therefore an important reservoir for these bacteria. In fact, each reptile should be considered as a *Salmonella* carrier until the contrary is proven. The classical biochemical methods for *Salmonella enterica* subspecies detection are time consuming, especially in samples from reptiles, because they frequently can carry more than one *Salmonella* subspecies. The aim of this study was to develop a multiplex PCR assay for *Salmonella* subspecies I, II, IIIa, IIIb and IV based on the detection of different siderophore genes. The application of biochemical siderophore-pattern analysis has indicated the existence of several iron supplying systems among clinical reptile and environmental *Salmonella* isolates. It has been known for more than 40 years that the major siderophore of *Salmonella* is the phenolate molecule enterobactin. Salmochelin, the long overlooked siderophore is a C-glucosylated enterobactin. Aerobactin, a hydroxamate siderophore is plasmid-coded found in some *Salmonella* subspec. I strains and chromosomal in strains of *S.* subspec. IIIa and IIIb. Furthermore, the operon of the siderophore yersiniabactin, was detected in *Salmonella* subspec. IIIb strains. *Salmonella enterica* also uses corynebactin via the IroN receptor and amonabactin via the Cir receptor. Furthermore, the gene *foxA* was selected for differentiation between *Salmonella* subspec. II and IIIa by PCR. In the present study, the occurrence of the 18 genes was examined in 41 reptile strains from the United Arabic Emirates by monoplex PCR. According to the results, a multiplex PCR assay was developed based on the genes *ttrCA*, *sciA, foxA, iutA*. Compared to biochemical analysis, this method allowed a fast identification of the subspecies from all Middle Eastern *Salmonella* strains (n=41), as well as 79 strains from German children (n=18). These results revealed the novel multiplex PCR as a fast assay for a specific identification of *Salmonella* subspecies I, II, IIIa, IIIb, and IV often associated with REPAS.

MMAP024

The role of tandem lipoproteins in *Staphylococcus aureus* USA300 and lipoprotein acylation in host signaling

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Staphylococcal lipoproteins are the dominant immunobiological active TLR2 ligands. Mature lipoproteins have been found to play a crucial role in staphylococcal invasion and persistence of infection. Yet, the knowledge of lipoproteins is still limited: the functions of only 35 among 50 genes containing a lipobox consensus sequence have been determined by either direct study or homolog sequence prediction.
We performed bioinformatical analysis on a tandem lipoprotein cluster located on the *Staphylococcus aureus* specific nSAa pathogenic island. The cluster shows a very high diversity in the different *S. aureus* clonal complexes, with *S. aureus* USA300 having one of the longest and most complex lipoprotein clusters. In order to reveal the function of the tandem lipoproteins in host signaling, we constructed a cluster deletion mutant as well as the complementary strain in *S. aureus* USA300.
The N-terminal structure of staphylococcal lipoproteins is still controversial: the degree of acylation (di- vs. triacylated) is supposed to make a considerable difference in innate immune response. Therefore, we overexpressed short lipopeptides consisting of only the signal peptide and the lipobox with different affinity tags. We were able to improve lipoprotein purification from staphylococci in order enable chromatographic and spectrometric analysis of lipoprotein modification in different pathogenic and apathogenic staphylococci.

MMAP025

Investigation of *Staphylococcus aureus* exoproteins with unknown function and their impact during infection

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Although *Staphylococcus aureus* is the predominant cause of nosocomial infections with increasing importance also for community-acquired infections, the mechanisms of host-pathogen-interaction and in particular the function of many virulence factors are still poorly understood. Regarding the genome sequence, the function of more than 60% of all predicted proteins is so far unknown. Interestingly, this includes a multitude of surface associated and extracellular proteins.
In this study, the function of selected exoproteins for the virulence potential of *S. aureus* and subsequently their role in host-pathogen interactions has been investigated. Therefore, putative secreted proteins with unknown function were overexpressed in *Escherichia coli* and purified. We were able to show an *in vitro* expression of some candidates in all investigated *S. aureus* strains. Furthermore, we found specific antibodies in mice sera after an *S. aureus* infection as well as in human sera of healthy donors. The results indicate an expression *in vivo* and suggest that these proteins may be important during infection. Next, we plan to examine the impact of interesting protein candidates on cells and components of the innate immune system especially the complement system.

MMAP026

Detection of antibiotics and antibiotic resistance genes in manures from different pig producing systems and fermentation residues from biogas plants

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Antibiotics are extensively used in animal livestock and often excreted unchanged or as metabolites by treated animals. These antibiotics lead to a fitness advantage of resistant bacteria present not only in the intestine of the animals but also in soil to which manures or fermentation residues are applied as fertilizers. In turn the abundance of resistant bacteria and of corresponding antibiotic resistance genes (ARGs) in the microbial community is assumed to increase. Via mobile genetic elements these genes can spread even among phylogenetically distant bacteria and new resistant genotypes of pathogens may emerge.
The aim of our project is to monitor antibiotics and ARGs in manures and fermentation residues in order to develop mitigation strategies and to minimize the risk of resistance transfer to pathogens.
To identify parameters influencing the degradation of antibiotics and the reduction of ARGs during storage of manure or its treatment in biogas plants we sampled 16 different pig producing facilities and 8 biogas plants (fermenters were fed with pig manure, one alternatively fed with bovine manure) at different processing steps. The samples were analyzed for the presence and content of antibiotics (via LC/MS/MS), ARGs and mobile genetic elements (via PCR, Southern blot and quantitative real time PCR).
The composition of the bacterial communities in manure from different farms and during the fermentation process of biogas plants were analyzed using DGGE of 16S rRNA gene fragments amplified from total community DNA. Similar dominant populations were observed for different manures while the bacterial community composition in samples from different stages of biogas plants was distinct.
Plasmids of different incompatibility groups (IncN, IncQ, IncP-1, IncW, IncU, Low GC), integrons containing gene cassettes of different sizes and several ARGs (*sul1*, *sul2*, *sul3*, *tetA*, *aadA1*) were detected in many of the samples. Our data indicate that not only piggery manures but also fermentation residues from biogas plants applied to field soils might contribute to spreading antibiotic resistance genes and mobile genetic elements.

MMAP027

Influence of aminocoumarins on gene expression, SOS response and adaptability in *Staphylococcus aureus*

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Aminocoumarins are specific inhibitors of gyrase B and were used to elucidate the role of DNA supercoiling. It was recently shown for *Streptococcus pneumonia* that genes responding to changes in level of supercoiling are organized in topology-reacting gene clusters [1]. In contrast to aminocoumarins, the widely used fluoroquinolones target gyrase A and/or parC and are known to induce the SOS response [2]. The SOS response has an important role for induction of mutation and thus accelerates the development of antibiotic resistance [2-3]. Here the influence of aminocoumarins on SOS response, mutation frequency and supercoiling mediated gene expression is analysed using wild type and mutant strains (*recA, lexA, arlRS,* novobiocin resistant strains).
We could show that novobiocin (an aminocoumarin) inhibits RecA expression and reduces the rate of recombination as well as the formation of heterogeneity alone as well as in combination with fluoroquinolones[3]. Microarray analysis revealed that novobiocin effects the expression of selected genes of different role categories. E.g. expression of the *recF-gyrA-gyrB* operon and the *rib*-operon was significantly increased, whereas expression of *recA* and some toxin genes *(hlgC, lukDE*) was decreased by novobiocin treatment for 5 min. This regulation was independent of the two-component system *arlRS* which was previously proposed to be involved in supercoiling sensing[4]. Further analysis using promotor activity assays in which the responsive genes were integrated into a different location in the chromosome revealed that supercoiling responsiveness of a given gene is independent of the genomic environment.

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MMAP028

Glycerol and Phospholipid Transporters in *Mycoplasma pneumoniae* - Implication in Growth and Virulence

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Mycoplasma pneumoniae is a pathogenic bacterium which colonizes the human respiratory tract and causes atypical pneumonia. Due to its longstanding close adaptation to the human host, the bacterium has evolved a minimal genome with only limited metabolic capabilities. Therefore, *M. pneumoniae* is strongly dependent on external nutrient supply and relies on efficient transport systems. Actually, about 17 % of the *M. pneumoniae* proteome account for transporters and lipoproteins that are involved in transport processes but whose function has so far not been explored. We investigated the role of several transporters and lipoproteins that are potentially involved in the uptake of glycerol and glycerophosphodiesters, the most abundant carbon source in the natural habitat of *M. pneumoniae*. Based on experiments with the corresponding mutant strains, we could demonstrate that the newly identified GlpP transport protein (MPN421) is responsible for the uptake of the glycerophosphodiester glycerophosphatidylcholine which is intracellularly cleaved to glycerol-3-phosphate. This uptake of glycerophosphatidylcholine is facilitated by the accessory transport proteins MPN076 and MPN077. Moreover, we found the lipoproteins MPN133 and MPN284 to be essential for the uptake of glycerol. Our data suggest that they act as high-affinity glycerol binding proteins which then deliver the glycerol molecules to the glycerol facilitator GlpF.

MMAP029

Identification of a periplasmic Aminoacyl-Phosphatidylglycerol Hydrolase responsible for *Pseudomonas aeruginosa* lipid homeostasis

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Specific aminoacylation of the phospholipid phosphatidylglycerol (PG) with alanine (or alternatively with lysine) was shown to render various organisms less susceptible to antimicrobial agents and environmental stresses[1,2]. In this study, we make use of the opportunistic pathogen *Pseudomonas aeruginosa* to decode PA0919-dependent lipid homeostasis. PA0919 is a homologue of AcvB/VirJ/AtvA former found responsible for plant infection[3] and acid tolerance[4]. Analysis of the polar lipid content of the deletion mutant ΔPA0919 indicated significantly enlarged levels of alanyl-PG. The impact of this artificially altered lipid composition was manifested in an increased susceptibility to several antimicrobial compounds when compared to the wild type. The recombinant overproduction of wild type and several site-directed mutant proteins in the periplasm of *E. coli* facilitated for a profound *in vitro* analyses of the enzymatic PA0919 function. A series of artificial substrates (p-nitrophenyl esters of various amino acids/aliphatic acids) indicated enzymatic hydrolysis of the glycine, lysine or alanine moiety of the respective ester substrates. Our final *in vitro* activity assay in the presence of radioactively labeled alanyl-PG then demonstrates that PA0919 codes for an aminoacyl-PG hydrolase that convertes alanyl-PG into alanine and PG.

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MMAP030

The Emergence of Novel Sequence Types of *Staphylococcus aureus* by Multilocus Sequence Typing

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Question: The rate of *Staphylococcus aureus* infection and dissemination in Pakistan are known to be high, but few studies have described the epidemiology of the different *Staphylococcus aureus* clones present. This study describes the epidemiology of *Staphylococcus aureus* within this region based on multilocus sequence typing (MLST) by analyzing sequence data obtained.
Method: A total of 1015 strains of staphylococcus were collected from tertiary care hospitals of the city. About 421 strains were confirmed as *Staphylococcus aureus* by characterizing them biochemically, out of which, 56 *Staphylococcus aureus* isolates were recovered for further analyses.
Results: All isolates were grouped into 39 sequence types (ST) and 5 clonal complexes (CC). Out of 39 STs, 22 STs were reported for the first time across the globe. Among all CCs, CC8 was found to be the most prevalent. The direct sequencing results exhibit significant changes at MLST loci showing point mutations that gave rise to new allele *aroE-343*, whereas *tpi-278* was a result of homologous recombination.
Conclusion: Therefore, this study concludes a high diversity in nature of locally circulating clones of *Staphylococcus aureus* and defining their geographic epidemiology.

MMAP031

Significance of *Legionella pneumophila* phospholipases C for modification of the *Legionella*-containing vacuole membrane

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Legionella pneumophila, the causative agent of Legionnaires' disease, replicates intracellularly within a compartment termed *Legionella*-containing vacuole (LCV). Bacterial phospholipases are important virulence factors, which for example modify the intracellular host environment. In addition to the manifold phospholipases A (eleven patatin-like, one PlaB-like, and three GDSL enzymes), *L. pneumophila* possesses three phospholipases C (PLCs), PlcA, PlcB, and PlcC. Sequence analysis revealed that these enzymes share conserved domains with the PC-PLC of *Pseudomonas fluorescens* and also with uncharacterized fungal proteins. PlcA and likely PlcB are secreted via the type II secretion system (Lsp) (Aragon et al. Microbiology 2002). In another context, PlcC was described as a yeast-cytotoxic type IVB-injected effector with unknown function and named CegC1 (Altman et al. Journal of Bacteriology 2008, Heidtman et al. Cellular Microbiology 2009). Only PlcC/CegC1 is conserved in all genomes of so far sequenced *Legionella* strains. Interestingly, however, all three PLCs are transcriptionally induced during host cell infection (Weissenmayer et al. PLOS One 2011; Brüggemann et al. Cellular Microbiology 2006). We hypothesize that the PLC proteins may hydrolyze LCV lipids and therefore function in remodeling and disintegration of the phagosomal membrane. We hence aimed to analyze the protein and lipid composition of the LCV following *L. pneumophila* wild type and PLC mutant infections. Further, purification of the PLC proteins and subsequent antibody generation were undertaken to determine PLC localization during infection.

MMAP032

Proteome analysis of the opportunistic pathogen Pseudomonas aeruginosa: comparison of different virulent strains

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The worldwide most common gram-negative pathogen *Pseudomonas aeruginosa* causes a wide range of syndromes in humans including chronic airways infections in patients with cystic fibrosis (CF) or chronic obstructive pulmonary disease (COPD). After analysis of the pangenome and virulence tests of 20 sequenced *P. aeruginosa* strains in standardized *in vitro* and *in vivo* infection models, four strains, (1) the most virulent (F469) and (2) the least virulent strain (B420), (3) an isolate of the most common cytopathic (C40A) and (4) cytotoxic clone (PA14) were chosen for further analysis. Here we demonstrate the first results of comparative 2D-gel-based proteome analysis of intra- and extracellular proteins of these three clinical isolates from COPD or CF patients (F469, C40A, PA14) together with those of one environmental isolate (B420), all grown in TSB medium. Interestingly, in spite of their different virulence, the exoproteomes of the broad host range strain PA14 and the least virulent environmental strain B420 seem to be very similar. For instance, both strains produce and secrete equal amounts of three types of proteases: (I) a common protease (protease IV), (II) an alkaline protease (AprA) and (III) elastase (LasB). Further commonalities and differences are shown and discussed. Furthermore we identified the complete intracellular protein sets of B420 and PA14. Thus, we could identify proteins up-regulated in the stationary phase, e.g. PA5535, a conserved hypothetical protein with similarity to cobalamin synthesis proteins, which is mostly induced in B420.

MMAP033

Characterization of the C-terminal region of *Legionella pneumophila* cell-associated phospholipase A PlaB: Importance for enzymatic activity and localisation at the bacterial surface

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The lung pathogenic bacterium *Legionella pneumophila* expresses a variety of phospholipases potentially involved in disease-promoting processes and development of pneumonia. The recently identified major cell-associated and hemolytic phospholipase A (PLA) / lysophospholipase A (LPLA) PlaB shares no homology to previously described phospholipases and therefore is the first characterized member of a new lipase family. So far, it was shown that PlaB utilizes a typical triad of Ser-Asp-His located within the N-terminal region of the protein for cleavage of phospholipids, such as phosphatidylglycerol (PG), -choline (PC), and respective lysophospholipids. We further determined that PC- but not PG-hydrolyzing PLA activity is directly linked to the hemolytic potential. PlaB also plays an important role as a virulence factor in the guinea pig infection model. However, till now the function of the C-terminal region of about 170 amino acids is unknown, but it is essential for lipolytic activity. Therefore, we aimed to characterize its function with respect to enzyme activity and localization. The analysis of three C-terminally truncated versions of PlaB revealed that a lack of only 5 amino acids (aa) leads to a decrease in PC-PLA activity, the lack of 10 aa results in a decrease of PG- and PC-PLA activity, and the removal of 15 aa completely abolishes enzymatic activity. Furthermore, an anti-PlaB antibody allowed detection of the protein at the surface and in the outer membrane fraction of *L. pneumophila*. Currently, analyses of protein regions and systems essential for PlaB export are on-going.

MMAP034

Cloning and expression of the genes qnrC and qnrD and functional analyses of their gene products as type-II topoisomerase protection proteins in E. coli

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Cloning and expression of the genes qnrC and qnrD and functional analyses of their gene products as type-II topoisomerase protection proteins in E. coli
Objectives: In recent years, an increasing number of plasmid mediated quinolone resistance (PMQR) mechanisms has been described, including novel efflux pumps (QepA, OqxAB), inactivating acetyltransferases (Aac6'-Ib-cr) and type-II topoisomerase protection proteins (QnrA). The Qnr-proteins feature a unique beta-helical structure that imitates DNA, thus binding to bacterial gyrase. The goal of the following studies was to investigate the role of two novel qnr-variants, qnrC1 and qnrD, in the quinolone resistance of various strains of E. coli with defined gyrase mutations associated with quinolone resistance.
Methods: The gene qnrD was isolated using PCR and subsequently cloned into pUC19 placing it under the control of the strong and inducible plac-promoter using deletion by inverse PCR. A strain of E. coli (WT) susceptible to fluoroquinolones (fq) and 6 isogenic derivatives carrying

known chromosomal mutations mediating quinolone resistance were transformed with the resulting recombinant plasmid pHPPH19 01.1 as well as with the plasmid pHS11 (qnrC). Subsequently the quinolone susceptibility was determined as minimal inhibitory concentration (MIC) for all recombinant clones for three different fq-antibiotics (CIP, LEV, MOX).
Results: MICs as well as the relevant mutations for the used strains are shown in the following tables

Stamm Mutation pHPPH19-01.1 CIP LEV MOX
WT + 0,064 0,256 0,256
- 0,016 0,032 0,032
MI GyrA S83L + 1 1 2
- 0,256 0,512 0,512
MII GyrA S83L, MarRΔ175bp + 2 4 4
- 1 2 2
WT-III MarRΔ74bp + 0,256 0,512 0,512
- 0,064 0,128 0,128
WT-3 GyrA S83L, GyrA D87G + 1 2 2
- 0,512 1 1
WT-3.2 GyrA D87G + 0,512 2 2
- 0,256 0,512 0,512
WT-4-M35 GyrA D87G, ParC S80I + 0,512 0,512 2
- 0,064 0,128 0,256
ATCC + 0,064 0,128 0,256
- 0,008 0,016 0,016

Conclusion: A strain-specific increase in MIC ranging from 2- to 16-fold was found. The genetic background of the strain had a mutation dependent impact on the increase in resistance : The higher the original resistance against fluoroquinolones was, the less noticeable was the effect of either resistance gene.

MMAP035

The Response of Staphylococcus aureus to Internalization into Human Cell Lines: a time resolved proteomics study

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S. aureus is a versatile Gram-positive pathogen that gains increasing importance due to the rapid spreading of resistances. It is responsible for a broad variety of local or systemic diseases and is one of the leading causes of bloodstream, lower respiratory tract and skin/soft tissues infections. Despite its capacity for survival and persistence in various tissues, S. aureus is classically considered as an extracellular pathogen. Nevertheless, it is able to internalize and survive within different non-professional phagocytic cells such as osteoblasts, epithelial and endothelial cells. In this study we comparatively profiled the proteomic adaption of S. aureus HG001 to the intracellular environment in human bronchial epithelial cells (S9), human alveolar cell (A549), and human embryonic kidney cells (HEK 293). Proteome patterns of internalized S. aureus cells isolated from 1.5 h to 6.5 h post infection were recorded by a workflow that combines cell sorting, on-membrane digestion, and mass spectrometry and the specific responses of S. aureus were quantified and compared. Changes in levels of regulatory proteins, induction of adhesins and toxins, induction of protection against oxidative damage and adaptation of cell wall synthesis have been detected in all three cell types. Moreover, glycolysis, PPP, and mureinsynthesis seemed to be uniformly up-regulated post-infection. Differences have been observed e.g. in the level of components of the translational apparatus and proteins encoded by the pur operon, which likely reflect differences in nutritional status which were also paralleled by different growth patterns of S. aureus after internalization by the different cell types. The comparison of the reaction of S. aureus to different human cell lines provides new insights into the adaptation network of S. aureus to different eukaryotic host cells after internalization.

MMAP036

The social amoeba *Dictyostelium discoideum* as a host model to study pathogenic and apathogenic yeast

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The social amoeba *Dictyostelium discoideum* naturally lives in forest soil and feeds on bacteria and other microorganisms. As professional phagocytes, the amoebae are used to study basic principles of phagocytosis. Furthermore *D. discoideum* has been established as a host model for several intracellular pathogenic bacteria like *Legionella*, *Mycobacterium,* and others. On the other hand little is known about the interaction between *Dictyostelium* and pathogenic and apathogenic yeast. Heat killed yeast are used to study phagocytic events but experiments with living yeast cells have not been conducted or failed so far.
Here we describe for the first time the interaction between *D. discoideum* with living pathogenic and apathogenic yeast. We modified a protocol originally established to study the interaction between *Acanthamoeba* and *Mycobacterium* to investigate the killing efficiency of *Dictyostelium* using different yeast strains. A *Saccharomyces cerevisiae* strain obtained from a local bakery turned out to be more resistant towards predation by the amoebae compared to commonly used lab strains. These data were confirmed by counting cfu after co-incubation of amoebae and yeast in a micro-well format.
Furthermore we investigated the interaction of *D. discoideum* with pathogenic *Candida* species. Here *C. albicans* is predation resistant producing hyphae after contact with the amoebae. *C. glabrata* however can be killed by *Dictyostelium*. As *D. discoideum* is highly amenable to genetic manipulation we also tested knock-out strains, which have been shown to play a role in phagocytosis and/or intracellular growth of pathogenic bacteria. Taken together we provide data that *D. discoideum* can easily be used as a host model to study pathogenic and apathogenic yeast.

MDV001

In silico evaluation of primer and primer pairs for 16S ribosomal RNA biodiversity

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16S ribosomal RNA gene (rDNA) amplicon analysis remains the standard approach for the cultivation-independent investigation of microbial diversity. However, the accuracy of these analyses depends strongly on the choice of primers. This issue has been addressed by an *in silico* evaluation of primer with respect to the SILVA non-redundant reference database (SILVA SSURef NR). A total of 175 primers and 512 primer pairs were analyzed with respect to overall coverage and phylum spectrum for *Archaea* and *Bacteria*. Based on this evaluation a selection of 'best available' primer pairs addressing different sequencing platforms is provided in order to serve as a guideline for finding the most suitable primer pair for 16S rDNA analysis in any habitat and for individual research questions. Moreover, the SILVA team developed a new SILVA TestPrime tool (http://www.arb-silva.de/search/testprime) allowing the scientific community to perform an online *in silico* PCR with their primer pair of interest.
Thereby (re)evaluation using an up-to-date database can be assured and evaluation of primer pairs prior amplifications remain attractive in the future.

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MDV002

Microbial communities involved in leaf litter degradation of annual and perennial plants

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Microbial degradation of plant litter material provides the primary resources for organic matter formation in soil. The aim of this study was to enlighten the role of bacterial colonisation on leaf litter fragments and to investigate shifts in microbial diversity during leaf litter degradation.
Therefore, we compared two different litter types: (I) *Zea mays* as annual and (II) *Fagus sylvatica* as perennial model plant. Leafs were sewed into nylon bags and incubated for up to eight (*Z. mays*) and thirty (*F. sylvatica*) weeks in the soil. The state of degradation was determined by the loss of dry weight. Additionally, the amount of sugars, amino sugars and phenols was analysed. Bacterial communities were profiled using 16S rRNA gene fingerprinting (T-RFLP). In order to characterise the composition of bacterial communities involved in litter degradation and to identify the key players, barcoded pyrosequencing of 16S rRNA gene amplicon libraries was performed.
In perennial leaf litter, chemical analyses revealed a higher amount of persistent substances as lignin and celluloses compared to annual leaf litter. The analysis of *Z. mays* litter showed shifts of microbial community. T-RFLP and the sequencing results nicely reflected concordant changes of the bacterial community over time. These results of *Z. mays* leaf litter indicated dominant bacteria shifting from Alpha- and Gammaproteobacteria towards Betaproteobacteria over time. In contrast, dominating Betaproteobacteria shifted towards Gammaproteobacteria on the leaf litter of *F. sylvatica*.
Thus, we conclude that the different r-strategists firstly metabolised the easy available low molecular nutrient sources such as polysaccharides and hemicelluloses, and the bacterial community subsequently changed over time following the gradient of higher molecular leftovers of persistent organic substrates.

MDV003

Multitrophic interaction between microorganisms, plants and herbivores: Does fertilizing, mowing or herbivory on plants alter the microbial community composition in the rhizosphere?

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Microorganisms play a major role in agricultural soils. They are functional for plant growth and health as well as for mediating the structure and dynamics of plant communities. These microorganisms maintain ecosystem functions through controlling nutrient cycling reactions and turnover of organic material.
The plant rhizosphere, defined as the soil layer surrounding the plant roots, is a dynamic environment in which many parameters influence the diversity, activity, and structure of microbial communities. Microorganisms living in this habitat form close mutualistic relationships with plants and benefit from nutrients provided by root exudates. Despite their importance for soil and plant health, the response of these microbes to different grassland management regimes and above-ground feeding is still poorly understood.
This study aimed at assessing and exploiting the soil microbial diversity with regard to plant diversity levels (monocot-enriched, dicot-enriched, and control), grassland management regimes (fertilization and mowing), and herbivory. Soil samples from a semi-natural, moderately species-rich grassland site near Silberborn (Solling; Germany) were collected in summer 2011 and further investigated employing different metagenomic approaches. To gain insights into the microbial community composition, total DNA was extracted from the samples and subjected to 16S rRNA gene PCRs. The resulting PCR products were either processed by DGGE analysis or sequenced via next-generation sequencing and further analyzed.
We recorded differences in the bacterial community structure with regard to the parameters mentioned above. Fertilization and mowing had a higher impact onto the bacterial community composition as compared to herbivory. Interestingly, community structures in monocot- and dicot-enriched plots were mainly driven by fertilization, whereas the bacterial communities in control plots were mainly influenced by mowing.

MDV004

Acidobacteria, *Bacteroidetes* and unclassi-fied Bacteria dominate the anaerobic degradation of cellulose in fen soil

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Cellulose is a major biopolymer in peat and peat soil-covering vegetation. Anaerobic degradation of cellulose and the associated microbial community was investigated in peat soil slurries derived from a moderately acidic (pH 4.5) fen. Propionate, acetate, methane, and carbon dioxide accumulated in anoxic ^{13}C-cellulose-supplemented soil slurries whereas solely methane and carbon dioxide accumulated in unsupplemented controls. The accumulation of organic acids rather than cellobiose or hydrogen in cellulose-supplemented soil slurries indicated that syntrophic fermentation was a bottle neck process in cellulose degradation. A 200% increase in electron turnover occurred in response to cellulose supplementation, indicating that poor availibilty of easily degradable organic compounds limited microbial activity in peat soil. Reduced methane production in cellulose-supplemented soil slurries might be caused by a combination of low pH and high concentrations of undissociated organic acids acting as uncoupling agents. 16S rRNA ^{13}C-based stable isotope probing indicated unclassified Bacteria, *Acidobacteria* (*Acidobacteriaceae* and *Holophagaceae*), and Bacteroidetes (*Porphyromonadaceae* and unclassified *Bacteroidetes*) as main anaerobic degraders of cellulose and cellulose-derived carbon sources in peat soil slurries. Members of the *Firmicutes* (*Clostridiaceae* and *Ruminococcaceae*) and *Spirochaetes* (*Spirochaetacaea*) were detected in lower numbers. 16S rRNA sequences affiliated to *Acidobacteriaceae* were up to 98% similar to *Telmatobacter bradus*, a facultative anaerobe recently isolated from peat soil and capable of cellulolysis. This indicates the presence of cellulolytic bacteria adapted to low pH, low temperatures, and low carbon availability in the fen. Other 16S rRNA sequences were closely affiliated to saccharolytic Bacteria (97% to *Paludibacter propionicigens*; 96% to *Spirochaeta zuelzerae*) indicating the contribution of saccharolytic bacteria to the overall degradation of cellulose. The collective data indicates that cellulose is readily degraded anaerobically to acetate and propionate by known and novel cellulolytic and saccharolytic fermenters in the fen.

MDV005

Biogeography of methane oxidizing bacteria: The β-diversity of *pmoA* genotypes in tropical and subtropical rice paddies

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Studies addressing microbial biogeography have increased during the past decade, however, research on microbial dispersal is still in its infancies and many aspects are only poorly understood. Here, we compared the methanotroph community in paddy soils sampled in Indonesia, Vietnam, China and Italy, focusing on the distance-decay relationship that is well known from the biogeography of macroorganisms. We used the *pmoA* gene as marker for methanotroph diversity in T-RFLP, microarray and pyrosequencing approaches. We could observe a significant increase of β-diversity with geographical distance at the global (12,000 km) and regional scale (20 km). Measured environmental parameters explained only a small amount of data variation and we found no evidence for dispersal limitation. Thus, we propose historical contingencies being responsible for the observed patterns. Furthermore, we performed an in-depth analysis of type II methanotroph *pmoA* distribution at the sequence level. We used ordination analysis to project sequence dissimilarities into a 3-dimensional space (multidimensional scaling). The ordination suggests that type II methanotrophs in paddy fields can be divided into five major groups, however, their diversity is widely distributed independent of the geographic

origin. No distance-decay relationship was observed at sequence level. By including tropical field sites (Indonesia and Vietnam) into the analysis, we further observed the first paddy fields harboring a methanotroph community depleted in type II methanotrophs.

MDV006
Microbial biofilms in a unique methane-fueled iodine-rich cave ecosystem

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Most subsurface ecosystems are limited by their supply of energy and carbon for microbes. Yet, cave ecosystems can harbor unique microbial life, isolated from photoautotrophic primary production, supported purely by in situ chemoautotrophic production. Here, we investigated the bacterial and archaeal community in an iodine rich spring cavern with high geospheric methane loading (up to ~1 % effective methane concentrations in the cavern atmosphere). The origin of the methane arises from highly concentrated gas emissions (>50% methane) within the spring water itself, confirmed by stable isotope analysis as natural, thermogenic gas (-41‰ $d^{13}C/^{12}C$). The massive biomass produced by the microbes in the form of biofilms and exopolysaccharide slime completely covers the walls and ceilings of the cave, the latter bearing bacterial snottites of over 10 cm length. A diverse microbial community within these biofilms is found, applying molecular fingerprints and in-depth 454 pyrotag sequencing, indicating a complex network of bacteria, archaea and microfauna within the sticky matrix. Sequencing results are currently still under way. We also assessed the diversity of methanotrophs amplifying the gene responsible for encoding the particulate methane monooxygenase enzyme (*pmoA*), present in most aerobic methanotrophs. Thus, new insights are gained into a unique subsurface ecosystem, independent of phototrophic energy and carbon inputs, but just several meters under our feet.

MDV007
Microbial community in anaerobic sediments of Tinto River determined by culture-dependent and culture-independent methods

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Tinto river (Huelva, Spain) is an extreme environment due to its acidic pH (mean 2.3), and high concentration of metals and sulfate, caused by the chemical and microbial oxidation of metallic sulfides from the Iberian Pyritic Belt. Here we describe the microbial community composition of anaerobic sediments of Tinto River using a combination of culture-independent and culture-dependent methods.
Culture-independent methods targeting the small subunit ribosomal RNA such as denaturing gradient gel electrophoresis (DGGE), 16S rRNA gene sequencing (cloning) and catalyzed reporter deposition fluorescence *in situ* hybridization (CARD-FISH) have been used to develop a geomicrobiological model of the different microbial cycles operating in the sediments. Microorganisms involved in the iron, sulfur, carbon and nitrogen cycles have been identified and their distribution correlated with physicochemical parameters of the sediments; where the pH and redox potential were closer to that of the water column (pH 2.5 and +400 mV), the most abundant organisms were identified as iron-reducing bacteria; while at higher pH (4.2-6.2) and more reducing redox potential (50, -210 mV), members of sulfate-reducing genera were dominant.
Additionally, to prove the occurrence of anaerobic microbial activities and to validate model with the presumed function of detected microorganisms, strategic enrichment incubations were made. Methanogenic, sulfate-reducing, denitrifying, iron-reducing and hydrogen-producing enrichments yielded positive results and the responsible microorganisms were identified.
Finally, classical techniques for bacterial isolation were applied and novel acidophilic sulfate-reducing bacteria (*Desulfosporosinus*/ *Desulfitobacterium* related), novel fermenters (very distantly related to *Paludibacter* and *Propionispora*) and hydrogen-producers (*Clostridium* spp.) were isolated. Additional test, such as the pH-range of growth, organic acids inhibition, H_2S effect and metal toxicity, are currently being performed with the isolates.

MDV008
Diversity and dynamics of bacterial populations in marine bioaerosols

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Question: To date, only little is known about marine airborne bacterial communitiesin terms of diversity, structure, composition and dynamics. Current studies showed that bioaerosols often featured similar bacterial communities as the underlying ecosystems. The primary mechanism for sea-to-air transfer of bacteria is the bubble-bursting mechanism at the ocean surface, passing the marine surface micro-layer which contains high concentration of microorganism. The sojourn time of microorganisms in air can amount to several days and even intercontinental transport is possible. Considering the consistent presence of pathogens in the native airborne community, it is crucial to analyse this special ecosystem due to public health concerns. Therefore, it is necessary to gain basic knowledge about the initial state and its natural fluctuations in order to detect “real” aberrations.
Methods: During a ship cruise from the North to the Baltic Sea in August 2011, 36 samples were taken using an impinger sampler (XMX/2L-MIL,Dycor, Canada). The aim was to analyse the species composition and to quantify bacteria in the marine air of different areas of the North- and Baltic Sea. The samples were analysed with culture independent molecular methods. Quantification was carried out using q-PCR and phylogenetic analysis was performed via 454 sequencing.
Results-Conclusion: First results showed a high variation in abundances of marine airborne bacteria ranging from $4.7*10^2$ to $1.1*10^5$ cells per m^{-3}. Per sample total numbers of 34 to 240 16S sequences were obtained. To integrate as much samples as possible the limit of sequences was set at 92 for the phylogenetic analysis. The results clearly showed a dominance of Proteobacteria (Alpha- and Gammaproteobacteria) with *Sphingomonas* sp. representing the most common bacteria. In addition, Firmicutes (Bacilli) were found in high abundances. The gathered results were integrated and correlated with the origin of air masses, previously calculated with backward trajectory modelling.

MDP001
Taxonomic characterization of antagonistic *Streptomyces* isolated from cultivated soils

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Methods: This study screened actinomycetes isolated from cultivated soils. A total of 341 actinomycetes isolates obtained from the different rhizospheric soils collected from Ngaka Modiri Molema district were screened for antibacterial activity. These potent bacterial isolates were characterized by conventional and molecular methods.
Results: Results indicated that 22.28% (76) of these isolates exhibited varying degrees of antibacterial activity against pathogenic Gram positive and Gram negative. The cultural, morphological and biochemical characterization of these potent isolates revealed that they belong to the genus *Streptomyces*. Molecular identification of the potent bacterial isolates of the 16S rDNA gene showed 89-100% similarities with *Streptomyces* spp. Phylogenetic analysis of the bacterial isolates based on the 16S rDNA gene sequencing supported their identification as *Streptomyces* spp. The sequenced 16S rDNA gene was submitted in the NCBI GenBank and accession number was assigned (JX860399-JX860432).
Conclusion: These results show a high diversity of *Streptomyces* associated with cultivated soils as well as their potential as antibacterial agents.

MDP003

Conceptualizing functional traits and ecological characteristics of methane-oxidizing bacteria as life strategies

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Methane-oxidizing bacteria (MOB) possess the ability to use methane for energy generation and growth, thereby, providing a key ecosystem service that is highly relevant to the regulation of the global climate. MOB subgroups have different responses to key environmental controls, reflecting on their functional traits. Their unique features (C1-metabolism, unique lipids, and congruence between the 16S rRNA and *pmoA* gene phylogeny) have facilitated numerous environmental studies, which in combination with the availability of cultured representatives, yield the most comprehensive ecological picture of any known microbial functional guild. Here, we focus on the broad MOB subgroups (type I and type II MOB), and aim to conceptualize MOB functional traits and observational characteristics derived primarily from these environmental studies to be interpreted as microbial life strategies. We focus on the functional traits, and the conditions under which these traits will render different MOB subgroups a selective advantage. We hypothesize that type I and type II MOB generally have distinct life strategies, enabling them to predominate under different conditions and maintain functionality. The ecological characteristics implicated in their adopted life strategies are discussed, and incorporated into the Competitor-Stress tolerator-Ruderal (C-S-R) functional classification framework as put forward for plant communities. In this context, type I MOB can broadly be classified as competitor-ruderal (C-R) while type II MOB fit more within the stress tolerator categories. Our literature review/meta-analysis shows that although MOB co-exist in the same environment, they possess distinct functional traits, reflecting on their life strategies, and may render a selective advantage under different conditions.

MDP004

Genotypic and functional charac-terization of culture-dependent endo-phytes from four common Taiwanese rice cultivars (*Oryza sativa* L.)

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Rice is an important crop species as more than 50% of human depend on it as their major food. Therefore, the sustainable and increased production of rice is an important matter. The production of rice not only is affected by environmental cues, such as soil, nutrients, sunlight, water, etc., microbial within (endophyte) or subsiding in the surrounding soil (rhizobacteria) also contribute considerably, such as increase in production and disease-resistance. In order to achieve our understanding of the culture-dependent microbiome in rice, which can be practically applied, this research selected 4 representative native cultivars (2 each of *Oryza sativa* subsp. japonica and *Oryza sativa* subsp. indica) to assess the differences in cultural microflora between the cultivars using the same soil media. By using the 16S rRNA sequencing approach, we have identified a total of 25 different rice endophytes that belong to the Firmicutes (44%), γ-proteobacteria (28%), β-proteobacteria (8%),α-proteobacteria (8%) and Actinobacteriae (12%). Amongst, 15 strains were identified as novel rice endophytes. A comparison between endophyte species in japonica and indica rice cultivars revealed that indica cultivars mostly consist of γ-proteobacteria (75%), while japonica cultivars are mostly Firmicutes (70%). This result suggests that endophytes in rice are cultivar-specific. Within the same rice subspecies, repeated identification of the same endophyte strains occurs mostly in the root. Functional characterization of the isolates showed 56% of the endophytes confer amylose dissolving capability, 36% confer cellulose dissolving capability, 76% confer protein dissolving capability and 16% confer calcium phosphate dissolving capability. These functional capabilities of the endophytes provide agricultural and industrial application potentials that merit further investigation.

MDP005

First steps towards reliable identification tools for marine fungi

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Fungi can be found in most ecosystems spanning from terrestrial to marine where they perform major functions as decomposers, parasites or symbionts. Overall fungal diversity is estimated to be 1.5 M species, but up to now only 70,000 species have been detected and described (Hawksworth 2001). It is expected that especially in the marine environment, several “hidden” species could be found with a high probability of forming new clades. With the breakthrough of next generation sequencing techniques, it is nowadays possible to describe true fungal diversity. However, here for the crucial step is the comparison of generated data with existing databases. The internal transcribed spacer region has recently been announced as the fungal barcode (Schoch *et al*. 2012). One drawback of this molecular marker is that due to its high sequence variability, it is not possible to construct a reliable alignments, which is the basis for phylogenetic analyses helpful in identifying new clades. One suitable region is therefore the ribosomal Small SubUnit (SSU). SILVA is a SSU sequence database downloading and filtering regularly fungal SSU sequences from the EMBL server (Pruesse *et al*. 2007). It contains in the current version 13,218 fungal sequences.
Aims of our project are, based on the SILVA database, (i) to construct a high quality alignment of fungal SSU sequences, (ii) identification of group specific variable regions, (iii) calculation of sequence diversity between taxonomic and ecological fungal groups and (iv) updating current knowledge of phylogenetic relation of marine fungi. This will serve as basis for environmental analysis of fungal communities and fungal group specific probe design.

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MDP006

Microbial community structure and function in the rhizosphere and drilosphere of subsoils

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Despite their importance for plant nutrition and other ecosystem services provided, subsoil systems have been rarely studied in the past compared to the topsoil compartment. It can be postulated that mainly in subsoils which are poor in nutrients and not disturbed by ploughing, the drilosphere and the rhizosphere are important structural and functional elements and have a high influence on the overall performance of B and C horizons of soils. Therefore, it was the aim of this study to investigate microbial community structure and function in different biopore systems of subsoils.
Samples from topsoil (10-30 cm) and subsoil (60-75 cm) were obtained at three distinct time points during the vegetation period from different field plots of an agricultural site in Klein-Altendorf (University of Bonn) and subdivided into bulk soil, drilo-, and rhizosphere. Bacterial communities were assessed using 16S rRNA gene fingerprinting. As a marker for microbial function the abundances of genes related to denitrification (*nirK*, *nirS*, *nosZ*) and nitrification (bacterial or archaeal *amoA*) were quantified by quantitative real time PCR.
Like expected there were pronounced differences in bulk soil in the bacterial community structure, as well as in the abundance of the measured functional genes between topsoil and subsoil samples. In contrast, in the drilo- and rhizosphere the afore mentioned differences between the two soil depths were not visible, and high gene copy numbers of the measured functional genes were also found in the subsoil samples. In the rhizosphere bacterial communities were mainly dependent on the plant species grown on the

sampled plot and showed only low similarities to bulk soil and drilosphere community structures.
As postulated, our results indicate that biopores in subsoil are of high importance as hotspots for nutrient turnover and differ in their function in relation to their origin.

MDP007

Influence of summer drought on the structure and function of microbial community in alpine grassland ecosystems

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The recent IPCC report predicts an increased occurrence of extreme weather events within the next decades with severe droughts in summer and heavy rainfall in winter. However, so far the influence of drought events on the microbial community in soils is largely unknown.
It was hypothesized that drought influences the structure and function of microbial communities to a large extend. To verify this hypothesis rhizosphere soil samples from two alpine grassland sites, which differed in their land use intensity were studied. To simulate drought, parts of the areas were covered with rain-out shelters which prohibited rainfall over a period of 9 weeks during summer. After the removal of the rain-out shelters the consequences of rewetting were studied for further 13 weeks. Samples of drought and rewetting as well as controls were taken at 8 different time points during the experiment. Soil water content, nitrate and ammonium contents were measured for all samples. Furthermore the abundances of different microbial communities involved in the microbial nitrogen cycle were determined based on the abundance of selected functional genes to investigate the impact of climate change on microbes involved in the nitrogen cycle. To analyze structural shifts in bacterial communities barcoded pyrosequencing of 16S rRNA gene amplicon libraries was carried out.
It could be shown that both sites differed in all measured parameters independent from the drought setting, with lower gene copy numbers and nutrient contents at the less managed site. An additional influence of the manipulation was mainly visible at the end of the drought period and during the first days of the rewetting period. Here also differences in the response pattern between the two sites were visible.

MDP008

Characterisation of the nitrifying community in various freshwater aquaculture systems

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Nitrogen is one of the most important elements of life and the biological conversion of ammonia via nitrite to nitrate is a key process in the global N-cycle. Nitrification is required wherever organic matter accumulates. The oxidation of ammonia to nitrate is catalysed by chemolithoautotrophic ammonia oxidizing bacteria or archaea (AOB / AOA) and nitrite oxidizing bacteria (NOB). The main biotechnological applications of public interest are wastewater treatment plants (WWTPs) and recirculation aquaculture systems (RAS), where the process of bacterial nitrification in special biofilters can still be regarded as black box. Nitrification is a very sensitive process, which is easily influenced by changing environmental parameters.
In this study, the nitrifying community structure of different freshwater RAS, which are distinguishable from each other in operational parameters (pH-value, temperature, fish population, feeding intensity) was analysed. The RAS used for the production of rainbow trouts was driven at a temperature of 13°C and pH 6.8. The other RAS used for the production of zander and carp were operating at a temperature of 23°C and a pH around 7. The nitrifying community was documented by electron microscopy, specific PCR just as cloning of the 16S rRNA- genes and FISH.
Although the analysed RAS were all supplied by the same water, varying operational parameters might affect the composition of the microbial population in complex ways. Since more than a decade it is known that *Nitrospira* is the key organism of nitrite oxidation in biofilters of aquaculture plants, whereas the presence of *Nitrobacter* seems to depend on a high substrate availability.
Analyses of the community structure revealed a varying coexistence of the NOB *Nitrospira*, *Nitrobacter* and the cold-adapted *Nitrotoga*, that was primarily found in permafrost-affected soils in Siberia, in the cold-water as well as in the warm-water system. In the biofilm samples of the rainbow trouts we could identify a member of *Nitrospira* with a similarity of 98% to *Nitrospira defluvii*.
Previous studies have shown that NOB are more sensitive relating to varying environmental parameters than the AOB. Up to now our results show that the AOB *Nitrosomonas* seems to be the main organism responsible for ammonia oxidation in all analysed RAS without exception.

MDP009

The genome sequencing of *Desulfotignum phosphitoxidans*

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Phosphorus occurs in biochemistry nearly exclusively as phosphates; reduced forms such as phosphite, hypophosphite or organophosphonates are considered to be only of minor importance, but can serve as P source for some bacteria in the absence of phosphate. The δ-Proteobacterium *Desulfotignum phosphitoxidans* oxidizes phosphite to phosphate as its only source of electrons, with either sulfate or CO_2 as electron acceptor to gain its metabolic energy.
The genomic DNA of *D. phosphitoxidans* was isolated using the MasterPure™ complete DNA purification kit (Epicentre, Madison, USA). 454 shotgun and paired-end libraries were generated. The *De novo* assembly was performed using the Roche Newbler assembly software 2.6 (Roche 454). For the gaps closure and sequence polishing, PCR-based techniques and Sanger sequencing of PCR products using BigDye 3.0 chemistry and an ABI3730XL capillary sequencer were used. The Gap4 (v.4.11) software was used for sequence editing.
The strain has 2 replicons, a single circular chromosome with a size of 4.88 Mb and a plasmid of 8 kb with an overall G+C content of the DNA 44.08 mol%. About 3664 (77 %) of the open reading frames (ORF) were assigned to functions. Two complete rRNA clusters and 47 tRNA genes including those for selenocystein incorporation were identified. Autotrophic CO_2 assimilation proceeds through the Wood-Ljungdahl pathway where ATP synthesis proceeds most probably via an acetyl-CoA synthetase (ADP-forming) instead of an acetate kinase 1]. In addition, the genome shows a restricted versatility and a high divergence in phosphonate and organophosphonate uptake and utilization systems in comparison with known systems in other Bacteria [2].
The genome sequencing provides important information on the composition and architecture of the phosphite-utilizing and energy-transducing systems needed to live on phosphite as unusual electron donor.

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MDP010

Inexpensive, sensitive and specific diagnostic assays for early detection of tuberculosis in cattle

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The present study was planned to estimate the sensitivity of the single intra dermal tuberculin test, dipstick assay, ELISA (using MMP, Cured culture filtrate ESAT-6, MBP70 and CFP10) and histopathlogical examination of the positive slaughtered animals for the detection of bovine tuberculosis. A total number of 1850 of cattle from different farms in Egyptwere examined for bovine tuberculosis (TB) by tuberculin intradermal test using bovine Purified Protein Derivative (PPD) prepared from *Mycobacterium bovis* (*M. bovis*). A total of 36/1850 (1.90%) were positive reactors by single cervical test. Histopathological examination of 155 lymph nodes and 69 organ tissues revealed that 37 lymph nodes and 19 organ tissue samples showed typical granuloma for tuberculosis. The collected samples (lymph nodes and tissue) from those showing visible lesions were showed positive isolations for *M. bovis* from 50 out of 96 samples (52%). ELISA assay using different antigens for skin tuberculin tested cattle (n=**60**: **24** Negative Reactor & **36** Positive Reactor) was increased to 61.67 and 63.33% by using ESAT-6 and MPB70, respectively. The dipstick assay was covered by the same antigens of ELISA assay. The sensitivity of the dipstick was ranged from 88.8 to

100%, while recorded 100.0% for ELISA. The Specificity of the dipstick was ranged from 87.5 to 100%, while recorded 91.6-100.0% for ELISA according to antigen type. In conclusion, the dipstick of the present study is inexpensive, sensitive and specific diagnostic assays for early detection of tuberculosis in cattle.

MDP011

Application of Propidium monoazide for live/dead differentiation of the bacterial community in raw milk

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The microbial diversity in raw milk depends on milking and processing procedures, hygiene standards, storage temperature and many other factors and is usually assessed by culture depended studies. These studies are based on the isolation of bacteria through growth on complex, synthetic or selective media. In addition, culture-based approaches are not able to detect slow growing organisms with complex or even unknown growth requirements or those who entered the viable but not cultivable state. These microorganisms can representatively be detected by direct molecular methods. But the use of DNA-based molecular detection tools for bacterial community analysis is impaired by the inability to differentiate signals originating from live or dead cells. The detection of the whole viable population is generally the most relevant factor for microbial safety and quality of food. Propidium monoazide (PMA) offers the possibility for selective removal of cells with damaged cell membranes from the analysis. The DNA intercalating dye forms an irreversible complex with extracellular DNA and DNA from dead cells after light exposure. Thus the PCR amplification of PMA-modified DNA is inhibited. For that reason a propidium monoazide assay for a selective molecular analysis of the viable bacterial community in raw milk was developed in our study. The study analyzed the viable bacterial population in raw milk samples from a farm bulk tank. We optimized the direct extraction of bacterial DNA from raw milk and combined this method with a live/dead separation through PMA. The bacterial communities of the bulk tank milk samples were characterized by analyses of their 16S rRNA genes from PMA-treated DNA.

MDP012

Spread of antibiotic resistance genes in plants and soil from wastewater-irrigated fields

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Wastewater (WW) contains a huge amount of potentially harmful compounds such as pharmaceuticals (e.g. antibiotics), resistance determinants and pathogenic bacteria. WW-irrigated soils and crops grown on fields irrigated by WW are a potential reservoir for multiresistant bacteria, which might pose risks on field workers and consumers of the agricultural products. The Mezquital Valley (60 km north of Mexico City) is the world´s largest WW irrigation area. There, untreated WW from Mexico City is reused for crop irrigation. In the Mezquital, fields have been irrigated with WW for different time periods from 1 year up to 100 years. Total DNA was isolated from several soil samples. From three different soil samples (non-irrigated, irrigated for 24 and 100 years) shot-gun sequencing of the bacterial 16S rRNA genes (V2-V3 region) was performed by pyrosequencing, to analyze the composition of the bacterial soil communities. The metagenomics data revealed a population shift of the bacterial community after WW irrigation. Around two fold more γ *Proteobacteria* (e.g. *Pseudomonas*, *Enterobacter*, *Legionella*, *Shigella*) were detected in the WW-irrigated soils, indicating a larger prevalence of potentially pathogenic bacteria in WW-impacted soils. In addition, *sul1* and *sul2* resistance genes encoding sulphonamide resistance and enterococci were quantified by real-time qPCR in the WW and in the different soil and plant samples from WW-irrigated and non-irrigated fields in the Mezquital. *sul* resistance genes and enterococci were present in high copy numbers (both between 10^3-10^7 gene copies/100 ml water) in the WW samples. *sul* gene concentration was three times higher, that of enterococci two times higher in the WW-irrigated soils (compared to non-irrigated soils), presuming also elevated concentrations of other resistance genes and pathogens in WW-irrigated soils. Thus, we hypothesize that there is an increased spread of resistance genes and resistant bacteria in WW-irrigated soils. Possible uptake of pathogens and resistance genes by herbs consumed raw was studied with herbs from fields in the Mezquital. Our first data show higher prevalence of enterococci and *sul* genes in herbs from WW-irrigated fields. An uptake of antibiotic resistant bacteria is likely and will be further studied to assess the risks for humans consuming these herbs.

MDP013

Identification of adapted rhizobia suitable for inoculation of legume plants in the Okavango region

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The Okavango Region is confronted with decreasing soil fertility and inadequate agricultural yields to support the rapidly growing population. To make up the deficit, local farmers use to expand their agricultural land into pristine areas. The application of diazotrophic bacteria may help to overcome nitrogen shortage and improve yields. Since local legume plants were found to be only sparsely nodulated, the aim of this study is to identify suitable rhizobial inoculants which are adapted to Namibian crops, climate and soil.

The isolation of root nodule bacteria was carried out from peanut (*Arachis hypogaea*), cowpea (*Vigna unguiculata*), Bambara groundnut (*Vigna subterranea*), common bean (*Phaseolus vulgaris*) and hyacinth bean (*Lablab purpureus*) plants growing under varying land management practices at different geographical locations in the Okavango region. The taxonomic position of the isolates was determined by BOX-PCR fingerprints combined with multi locus sequence analysis (MLSA) using concatameric sequences of the 16S rDNA and ITS as well as the *recA* and *glnII* genes. Additionally, phylogenetic relationship of *nifH* genes was analyzed. Host-range and nodulation efficiency of rhizobial isolates was investigated by inoculation studies and acetylene reduction assays (ARA). Adaption to dry Namibian climate was assessed by tests for resistance to drought and tolerance of high temperatures.

First results highlight that bradyrhizobia are the predominant root nodule bacteria which can be isolated from Okavango legume crops with their relatedness rather depending on geographical origin than on host-plant. As many isolates were able to grow at high temperatures of up to 39.5°C and showed the ability to outwear periods of drought, indigenous rhizobia seem to be well adapted to Namibian climate.

MDP014

Analysis of the microbial nitrogen cycle in the Okavango basin of southern Africa for sustainable land use management

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In the savanna ecosystems of southern Africa, nitrogen resides mainly in soil organic matter and limits plant productivity. Consequently, microbial mineralization of soil organic matter is a crucial factor for plant nutrition and soil productivity. To date little is known about the relation between soil management practices and microbial nutrient cycling in savanna soils. To fill this gap, we are studying key transformations of the microbial nitrogen cycle (focusing on ammonification, denitrification and biological nitrogen fixation) from soils of the Okavango region, particularly from Namibia, Angola and Botswana. Different land use strategies are included, such as pristine riparian forests, fallows, bushvelds, dryland farming and irrigation farming.

We applied cultivation-independent techniques based on functional marker genes for ammonia oxidation (*amoA*, encoding the alpha subunit of the ammonium monooxygenase), denitrification (*nirK* and *nirS* encoding for nitrite reductases) and nitrogen fixation (*nifH*, encoding Fe-protein of nitrogenase). DNA and mRNA extracted from soils are used as target molecules and subjected to PCR or RT-PCR, respectively. We also determined nitrate concentration of soils and measured the potential of N_2O emission there. We aim to identify the processes in which the bacterial communities are active and also to measure their changes in response to different land use management strategies. Furthermore, we will measure the levels of mRNA by qRT-PCR as a molecular estimate of bacterial activity.

Our initial results show that RNA extracts from pristine riparian forest soils contain *nirS* and archaeal *amoA* mRNA levels detectable by RT-PCR. Additionally, the same type of soils also presented the highest concentrations of nitrate and N_2O emission potential. In all other types of

soils similar microorganisms were present but not active. The analysis of all soils will give a better overview of how the nitrogen cycling microbial communities behave in these locations and give a hint on how they contribute to the agricultural productivity of savanna soils.

MDP015

Estimation of bacterial community in the Bokakotorska Bay (Montenegro) by card fish method

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The Boka Kotorska bay is located in the southeastern part of Adriatic sea comprising a part of Montenegrian coast. Geographically and oceanographically the bay represents a closed basin with specific hydrographic and hydrodynamic characteristics. The bay is composed of several smaller bays. The aim of this study was to estimate bacterial community in this specific enviroment. Our data provide the first insight of Boka Kotorska bay bacterioplankton structure.
Samples were collected at 10m of depth in June of 2011 and February of 2012 in the following bays: Kotor, Risan, Tivat, Herceg Novi bay and Mamula. (entry point of Bokakotorska bay). The bacterioplankton community was analysed by catalyzed reporter deposition fluorescence in situ hybridization with a set of rRNA targeted oligonucleotide probes. Bacteria were estimated with the general probe mixture (EUB I-III), *Gammaproteobacteria* (GAM42a), *Bacterioidetes* (CF319a) and *Alphaproteobacteria* (Alpha968).
Total bacterioplankton abudance as determined by DAPI counting varied from $0.2x10^6$ cell mL^{-1} to $1.3x10^6$ cell mL^{-1} (in June) and $0.4x10^6$ cell to $1.5x10^6$ cell mL^{-1} (in February). *Bacteria*, as detected by the probe mix of EUB338 I-III, dominated the microbial community (on average 80% of DAPI stained cell in June and 60% in February). The highest relative abundance of *Gammaproteobacteria* (42%) was detected in Kotor and Risan bay and the lowest in Mamula (5%). Similar situation were obtained with other groups of bacteria: The highest number of *Alphaproteobacteria* (25%) and *Bacterioidetes* (22%) were detected in Kotor bay and the lowest (5%) in Mamula. Hidrography of Kotor and Risan bay is characterized by frequent inflow of fresh water and organic load. Due to this inflow of water in Kotor and Risan bay, an increase of all gropus of bacteria was found.
Futher investigation will be based on more frequent sampling and using other oligonucleotide probes.

MDP016

Impact of transient flow conditions on bacterial community composition as revealed in 1D sediment columns

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Groundwater is generally perceived as an environment with relatively stable conditions for bacterial communities. However, at the interface between unsaturated and saturated zone transient abiotic conditions, like changes in groundwater recharge and mass fluxes, are hypothesised to be a hotspot for bacterial community dynamics. This may influence in turn ecosystem services provided by groundwater microbes. Thus, the bacterial community composition was investigated in two column experiments under steady-state and transient flow conditions (length = 50 cm, diameter = 9 cm). The water table of one column remained static, while transient flow conditions were simulated in the second column by actively altering the water table over ~25 cm in 48 h intervals. The columns contained a natural sediment matrix and were fed with oligotrophic groundwater. After 6 months, both columns were infiltrated with an artificial DOC influx (mixture of R2A-medium, acetate and humic substances, the resulting DOC content was tenfold higher than that of the natural groundwater) in order to study the effects of static and transient flow conditions on sediment bacteria and their activities. There were no observable geochemical distinctions between columns for sampled pore water, and only minor hydrological differences such as an elevated mean transit time in the column with transient conditions. The degradation of the amended DOC happened in both columns at the unsaturated sediment surface, and neither in the underlying capillary fringe nor in deeper sediments. Yet, depth-resolved T-RFLP analysis of the bacterial community revealed clear distinctions in bacterial community composition under transient conditions compared to the static setting. While most components of the bacterial community were present in both columns, several populations were clearly more abundant and more widespread under transient flow conditions over the entire fluctuation zone. These results demonstrate the susceptibility of bacterial communities to transient groundwater hydraulics. The observed community changes under transient flow conditions may have ecological implications on natural attenuation and bioremediation scenarios in groundwater, by enhancing reactive zones and filter functions at interphases.

MDP017

Succession of microbial communities during biological soil crust development on inland dunes

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Intensified agriculture in combination with climate change and the increased occurrence of extreme weathering or glacier retreats lead to an increase of vegetation free areas. Especially at initial stages of ecosystem development, succession is accelerated at biological hotspots like biological soil crusts (BSC). These are characterized by enhanced microbial activities, which change the nutrient content and water fluxes in soil, increase resilience of soils and form the substructure for future stages of succession. With ongoing succession, the function and composition of biological soil crusts change, starting with light, thin cyanobacterial dominated crusts via intermediate stages to dark, thick crusts dominated by mosses. While the general properties of BSC are often investigated, changes in the molecular structure and microbial network at different stages of BSC development are poorly understood. Therefore, it was the aim of this study to compare changes of the microbial community composition of BSC from initial, intermediate and developed BSCs.
Samples were taken at the Lieberose inland dunes in north-east Germany. There, BSC appear along a gradient from the mobile part of sand dunes towards the parts that are stabilized by the grass *Corynephorus canescens*. In the mobile part, the BSC are in the initial stage (thin, brittle, mostly cyanobacteria), while developed dark BSC (algae, mosses, thick crust) appear between the grass patches. Samples of BSC from three development stages and the bare substrate were compared by terminal restriction fragment length polymorphism (tRFLP) analysis of the 16S rRNA gene and barcoded pyrosequencing of 16S rRNA gene amplicon libraries. Additionally, BSC were characterized by determining the chlorophyll content and the water repellency index.
The number of dominant tRFs (> 1%) was highest in samples obtained from the intermediate BSC development stage, whereas all other samples showed similar amounts of dominant tRFs. Despite comparable amounts of dominant tRFs, principal component analysis revealed distinct bacterial communities at each BSC development stage indicating a shift in the dominant microbial community. To figure out the key players and describe changes in the microbial networks during BSC development data of 16S rRNA gene amplicon libraries will be presented.

MDP018

On the trail of thermophilic nitrifiers from hot springs of Yellowstone National Park

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By now, the only cultured thermophilic ammonia oxidizing representative, growing at 72°C, is the archaeon '*Candidatus* Nitrosocaldus yellowstonii'. Known thermophilic nitrite oxidizers belong exclusively to the genus *Nitrospira*, but the identity of nitrite oxidizers growing above 60°C is still unknown. In this study biofilms and sediments from two springs in Yellowstone National Park (USA) were used for the selective enrichment of thermophilic ammonia- and nitrite oxidizers.
Growth curves, performed with different enrichments at 70°C, confirmed the successful cultivation of ammonia oxidizers. Microscopic investigations showed the existence of coccoid cells as well as rods in these cultures. Analyses of the 16S rRNA genes revealed the occurrence of *Nitrosocaldus yellowstonii* in the initial enrichment culture as well as other nitrifying *Thaumarchaeota* (85-98% similarity). In accordance, the archaeal ammonia monooxygenase gene (*amo*A) was detected in a first enrichment. Clone analyses of a further enrichment gave hints for the presence of *Thermomicrobium roseum* (98-99%), *Ammonifex degensii* (84%), relatives

of *Sphaerobacter thermophilus* (82-88%) and *Thermobaculum terrenum* (82%). Additional immunofluorescence labeling of rods and filaments with polyclonal antibodies targeting the bacterial AmoB revealed the existence of ammonia oxidizing bacteria.
Next to the consumption of ammonia, it was detected that in two ammonia-oxidizing cultures simultaneously a turnover of nitrite to nitrate occurred. From these cultures, we successfully enriched nitrite oxidizers in mineral salts medium. The dominant morphotypes were short rods, which do neither react with the general bacterial (Eub 338I) nor the archaeal (Arch 915) oligonucleotide probes. Furthermore, flexible rods could be observed, which were identified as *Thermoleophilum album* by 16S rRNA gene analysis (99%). Short rods grow at 65°C and differ obviously from *Nitrospira* in cell shape, but their identity remains unknown until now.
Currently, both ammonia and nitrite oxidizing enrichments originating from Yellowstone Nation Park grow very slowly at 65°C. Nevertheless, in this study we could present a first glimpse of the diversity of thermophilic nitrification.

MDP019

First insights into symbiotic diversity in deep-sea wood-boring clams

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Sunken wood provides high quality carbon to the sea floor in both shallow and deep sea waters. These organic falls are rapidly colonized by specialized organisms such as wood boring bivalves which degrade the wood by digestion. Deep-sea wood boring bivalves (Family: Pholadidae; Subfamily Xylophagainae) occupy the same ecological niche as their shallow water counterparts, the so called shipworms (Family Teredinidae). Both groups harbor symbiotic bacteria in their gills. While shipworm symbionts have been identified as cellulolytic nitrogen fixing gammaproteobacteria, very little is known about the symbionts of Xylophagainae.
This study focuses on the diversity and biogeography of symbionts of deep water Xylophagainae. We present here preliminary results of symbionts of five species of this subfamily which were recovered with wood logs that had been experimentally deposited in 600-2300 m water depth at several locations in the deep eastern and western Mediterranean, the adjacent Gulf of Cadìz in the northeastern Atlantic and at the Rainbow hydrothermal vent field on the Mid-Atlantic Ridge. Initial phylogenetic characterization of the symbionts based on 16S rRNA clone libraries revealed that the majority of the clone sequences clustered with those of Teredinidae symbionts suggesting that they are also cellulolytic and fix nitrogen. We regularly found two or more different symbiont phylotypes in single host individuals. Some host species contained closely related symbiotic phylotypes across geographical locations indicating host specific symbiont distribution. Other phylotypes were more closely related among different host species co-occurring at the same location indicating geographic structuring of symbiont distribution rather than host specificity. Analyses of symbiont clone libraries from additional host individuals and also fluorescence in situ hybridization analyses with phylotype specific oligonucleotide probes will clarify this question in the future.

MDP020

Aerobic methanotroph diversity and *pmoA* gene transcription in Lake Stechlin sediment

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Methane is an important greenhouse gas whose atmospheric concentration has strongly increased in the last 250 years. Methanotrophs can use methane as their sole source of both carbon and energy and play an important role in mitigating methane release from soils and sediments. Known mesophilic aerobic methanotrophs belong to the *Proteobacteria*. Methanotrophs are typically termed Type I (*Gammaproteobacteria*) and Type II (*Alphaproteobacteria*). The particulate methane monooxygenase (pMMO) is a key enzyme for methanotrophs and the *pmoA* gene, encoding a subunit of pMMO, has been widely used as both a functional and phylogenetic marker for these organisms. Recent studies have also used transcription of *pmoA* as a proxy for methanotroph activity. The objective of the study was to investigate the diversity of *pmoA* genes in depth profiles of Lake Stechlin profundal sediment and to monitor *pmoA* transcription at different depths in the sediment. Quantitative PCR assays of *pmoA* genes indicated that methanotrophs could be detected to at least 40 cm depth; however, the *pmoA* abundances were 1 to 2 orders of magnitude lower than at the sediment surface. Analysis of *pmoA* genes by terminal restriction fragment length polymorphism (T-RFLP) approach indicated a diversity of both Type I and Type II methanotrophs. A particular clade of Type Ib sequences was relatively more abundant at depths below 5 cm than in the uppermost layer of the sediment; however, *pmoA* transcripts from this clade were always relatively low. Oxygen has been shown to penetrate only the top 1-2 cm of Lake Stechlin profundal sediment, therefore methanotrophs should be active only in this top horizon. The *pmoA* transcripts were quantified by reverse transcription and qPCR. The highest transcript abundances were in the top 2-3 cm, but transcripts were still detected at depths of 20 cm.

MDP021

Who's there and what are they doing? - Analysis of the microbial composition and their potential functions in photobioreactors

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Studies regarding the development of biofilms of microalgae and the interaction between prokaryotic and eukaryotic microorganisms are very limited. In the analyses presented here, the development of biofilm and the bacterial community of the microalgae *Scenedesmus* sp. and *Chlorella vulgaris* were examined in detail over a time period of three month in a reactor. The diversity and population dynamic of the bacteria were examined through analyses with scanning electron microscope (SEM), fluorescence in-situ hybridization (FISH) and 16S rRNA gene analyses (e.g. denaturating gradient gel electrophoresis). Microscopic examinations showed various cocci and rods of different sizes (0.5-2 µm) and shapes. Their diversity ranges from ball-shaped to chains of balls (cocci) and cylindrical with ends more or less rounded (rods). Moreover, various appendages and suctorial organs are visible. Biomolecular analyses indicated that various populations of alpha- and betaproteobacteria (concerning the family of Comamonadaceae) as well as bacteroidetes (e.g. *Pedobacter*, *Sediminibacterium*, *Flavobacterium* and uncultured Bacteroidetes) are associated with the microalgae examined here. However, the populations of alphaproteobacteria (e.g. *Sphingomonas*, *Brevundimonas*, *Sinorhizobium*, *Arcicella* and *Ochrobactrum*) as well as the populations of bacteroidetes dominating. Altogether the diversity is rather limited. To further analyse the algae-bacterial interaction, the near complete metagenome of this microbial community was established using Illumina and FLX 4/5/4 sequencing. These results imply the hypothesis that metabolic performance of the bacterial populations is probably related to the growth and stability of the algal culture. Moreover, the current work is focused on transcriptome analysis.

MDP022

Fast microbial community response and development of aerobic and anaerobic degrader populations upon toluene contamination of a pristine indoor model aquifer

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An indoor aquifer mesocosm system was used to assess, for the first time, the impact of toluene contamination on natural microbial communities of a pristine aquifer system, and degrader succession during the onset of natural attenuation processes. For reasons of historic contamination, these processes are mostly unobservable in the field. After introduction of a moderate toluene contamination, sediment and groundwater of the indoor aquifer were analysed and compared in 2D and over time. A rapid development of abundant aerobic toluene degraders as shown by toluene oxygenase (*tmoA*)

genes (up to 2.1 x 10^6 genes mL^{-1} groundwater and 1.5 x 10^6 genes g^{-1} sediment) was found only 16 days after contamination over the whole plume as active zone. Highly significant differences in the microbial communities between the sediment and the groundwater (R = 0.73, p = 0.0001) were revealed via T-RFLP (terminal fragment length polymorphism) and sequencing. Pyrotag sequencing revealed an immediate response towards the toluene plume via a selection of *Pseudomonas* spp. populations as the primary aerobic degraders, with successional dominances also of *Zoogloea* spp., *Dechloromonas* spp., unclassified *Comamonadaceae*, but also *Bdellovibrio* spp., hinting even at an evolving toluene-based microbial food web in the aquifer. Under secondary contamination with high toluene loads (max. 100 mg L^{-1}), another clear change in overall and degrader communities was observed, with denitrifying hydrocarbon degraders related to *Azoarcus* spp. appearing in the highly-contaminated sediment, coupled to spatial shifts of degraders within the *Pseudomonadaceae* towards the plume fringes. These findings provide unique insights into the reaction of pristine aquifer microbiota to contamination, and the spatial and temporal development of degrader populations adapted to local contaminant and redox scenarios.
In sum, it could be show how ongoing reactive processes and involved degrader populations evolve simultaneously, adapting spatially to local contaminant and redox scenarios but staying highly variable in groundwater.

MDP023
Isolation of rhizobacteria from salt tolerant plant species and evaluation of their plant growth-promoting ability

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Rhizosphere samples of *Hordeum secalinum* and *Plantago winteri* were taken from a salt meadow in Hessen, at each season of the year. The microbial community was analysed by culture-dependent methods with emphasis on diazotrophs (NFB & LG agar), phosphate- and phytate-mobilising bacteria (CP & IHP agar), ACC (1-aminocyclopropane-1-carboxylate) deaminase-active bacteria (DF agar) as well as IAA (indole-3-acetic-acid)-producing bacteria (LBT agar). Results showed that the bacterial cell numbers of each functional group differed from season to season and between plant species confirming that plant species and abiotic factors have an impact on the dynamic and structure of the rhizobacterial community. Most of the 119 isolated strains belong to the diazotrophic and the P-mobilising bacteria. The partial 16S RNA gene sequence of the bacteria showed that they belong to the *Proteobacteria*, *Actinobacteria,* and *Firmicutes*. Among the diazotrophic isolates new species belonging to the genus *Cellvibrio* and *Rheinheimera,* and a new genus belonging to the order *Rhizobiales* have been found. These strains are currently evaluated for plant growth-promoting ability (PGPA) under salt stress concentration (5 dS m^{-1}) using summer barley (*H. vulgare*, cultivar Morax) in greenhouse experiments.

MDP024
Enrichment and characterization of anaerobic naphthalene degrading microorganisms

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Polycyclic aromatic hydrocarbons (PAHs) are pollutants of great concern due to their potential toxicity, mutagenicity and carcinogenicity. PAHs are widely distributed in the environment by accidental discharges during the transport, use and disposal of petroleum products, and during forest and grass fires. Caused by their hydrophobic nature, PAHs basically accumulate in sediments from where they are slowly released to the groundwater. Although generally limited by the low water solubility of PAHs, microbial degradation is one of the major mechanisms leading to the clean-up of PAH-contaminated sites. Whereas organisms and biochemical pathways responsible for the aerobic breakdown of PAHs are well known, anaerobic PAH biodegradation is less good understood; only a few anaerobic PAH degrading cultures have been described.
To study the anaerobic PAH degradation in more detail a microcosm study was performed to enrich anaerobic PAH degraders. Therefore anoxic groundwater and sediment samples were used as inoculum. Groundwater samples were purchased from an erstwhile gas works and a former wood impregnation site. In contrast, sources of sediment samples were a former coal refining area and an old fuel depot. Finally, we established six enrichment cultures able to degrade naphthalene under sulfate reducing conditions. All were characterized by 16S rDNA gene sequencing and global proteome analyses. First results point to a dominance of identified sequences affiliated to deltaproteobacterium N47, which is a known anaerobic naphthalene degrader, in all enrichments. The abundance of this organism at different biogeochemical environmental sites will be discussed.

MDP025
Comparison of particle-associated microbial communities in limnic and marine systems

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Organic aggregates and associated microorganisms play a crucial role for organic matter cycling in both freshwater and marine systems. As hotspots for microbial activity they harbor microorganisms which may be underrepresented in the water column. In this study, we compared particle-associated microbial communities in freshwater (Lake Stechlin and Grosse Fuchskuhle) with those in marine systems (North Sea (Kabeltonne) and Northern Adriatic Sea). Semi-automated analyses of ~3,000 particles by motorized microscopy showed that in stratified systems particles above the thermocline are larger, but particles in limnic and marine system do not systematically differ in size. However, cell number correlation with particle size was better in limnic than in marine systems. This suggests that colonization of particles, especially in marine systems, is determined by additional factors than solely availability of space. Individual particles vary significantly in their bacterial communities as identified by fluorescence in situ hybridization (FISH), thus often encountering in one sample a range between 0 to 100 percent colonization by a respective taxon. This suggests that particle type, bacterial source community and bacterial succession significantly determine microbial communities on the particles. We performed pyrosequencing for the 16S rRNA gene, of limnic and marine samples from different depths and seasons. The analysis of ~330,000 sequences revealed that the communities on particles are diverse, but distinct and vary greatly in time and space. In limnic systems, particle-associated bacterial communities were dominated by Alpha- and Betaproteobacteria. The latter is less abundant in Lake Stechlin than in Lake Gross Fuchskuhle. Actinobacteria were occasionally found as attached in large numbers in Lake Grosse Fuchskuhle. The Gammaproteobacteria group was periodically found as attached and as free-living in both lakes. In marine systems, Alpha-, Beta-, Gammaproteobacteria and Bacteroidetes constituted similar fractions on particles. These results show that bacterial community composition on individual particles is highly variable and thus depend on individual particle features both in limnic and marine systems.

MDP026
Increasing biodiversity of PAH degrading Bacteria improves Bioremediation processes in contaminated soils.

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Soil contamination by polycyclic aromatic hydrocarbons (PAHs), a byproduct of industrial activities, is a worldwide threat for environmental safety and human health. PAHs are toxic, carcinogenic, and accumulate in soils. Pollutants may be efficiently removed by using so-called biodegradation approaches that use the potential of microorganisms to naturally degrade pollutants. Many organisms have been isolated that can degrade PAHs, and the underlying enzymatic pathways are now well described. However, attempts to use such organisms in bioremediation of polluted soils failed so far, to a large extend because of the lack of knowledge on the ecological parameters driving the survival and activity of these organisms. These limitations can be widely overcome by considering pollutant degrading microbes not only as a closed entity, but rather as the part of a complex and dynamic soil community. Especially, biodiversity has

often a positive effect on the performance and stability of microbial communities.
In this study we investigate if species richness of PAH degrading communities improves bioremediation. We set up defined bacterial communities containing one to 16 species able to degrade PAHs, and followed Phenanthrene degradation in soil microcosms. Biodiversity increased biomass, enzymatic activity and Phenanthrene degradation. This enhanced performance was largely driven by species with a low performance in monoculture, but that were able to stimulate biodegradation processes in diverse communities. Our results provide new ways to develop new efficient strategies to decontaminate soils.

MDP027

Intraspecific diversity increases plant-growth promotion by rhizosphere microbial communities.

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Root-associated bacterial communities are important drivers of plant health and productivity. Bacteria produce extracellular enzymes such as chitinases or proteases that break down organic matter and make nutrients available for plants. Rhizosphere bacterial communities are diverse. Diversity may increase ecosystem functioning via complementarity interactions, but increased diversity may also drive antagonistic interactions between competitors. We hypothesized that biodiversity drives the effect of rhizosphere *Pseudomonas fluorescens* on nitrogen uptake of *Arabidopsis thaliana*. We inoculated plants with defined bacterial communities ranging from one to eight genotypes. Plants were grown on a minimal medium containing casein as sole nitrogen source, so that plants depended on bacterial proteolitic activity for N uptake. We measured plant growth, N content, as well as the density and protease production of the bacterial communities. Increasing bacterial diversity enhanced plant performance. This effect correlated to a higher proteolitic activity of the bacterial communities, suggesting that competition among bacteria result in an increased nutrient mineralization. Interestingly, root colonization decreased with diversity, suggesting that root-associated pseudomonads inhibit competitors. Our results show that diversity of root-associated microbial communities is central for of nutrient mineralization and plant performance. We therefore suggest that diverse communities of plant growth-promoting bacteria may increase plant performance much beyond the effect of single species.

MDP028

Sequencing Orphan Species initiative (SOS): filling the gaps in the 16S rRNA gene sequence database for all species with validly published names

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High quality 16S ribosomal RNA (rRNA) gene sequences from the type strains of all species with validly published names as defined by the International Code of Nomenclature of Bacteria is a prerequisite for their accurate affiliations within the global genealogical classification and for the recognition of potentially new species. During the last few years the Living Tree Project (LTP, http://www.arb-silva.de/projects/living-tree) has taken care to create a high quality, aligned 16S and 23S rRNA gene sequence database of all type strains. The manual curation of the sequence dataset and type strain information revealed that in total 552 "orphan" species (about 5.7% of the currently classified species) had to be excluded from the reference trees. Among them, 322 type strains were not represented by a SSU entry in the public sequence repositories. The remaining 230 type strains had to be discarded due to bad sequence quality. Since 2010, the LTP team coordinated a network of researchers and culture collections to improve the situation by (re)-sequencing the type strains of these "orphan" species. Here we report 351 16S rRNA gene sequences of type strains. 201 species could not be sequenced because either cultivable type strains were not available (121), or the cultures had either been lost or were never deposited in first place (66), or due to other constraints (14). The International Code of Nomenclature of Bacteria provides a number of mechanisms to deal with the problem of missing type strains and we recommend that due consideration be given to the appropriate mechanisms to help solve some of these issues.
SOS Consortium partners: ATCC, BZF (at University of Hamburg), CECT, CIP, CCUG, DSMZ, JCM, ICMP, BCCM/LMG, NBRC, NCCB, and LTP team.

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MDP029

Diurnal pattern of bacterial activity and growth in a humic lake

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Solar radiation causes photochemical reactions that lead to the breakdown of dissolved organic matter in aquatic ecosystems. This process enhances the availability of low molecular weight substrates for heterotrophic bacteria. Simultaneously humic substances act as photosensitizers and mediate the formation of reactive oxygen species (ROS). Therefore, bacteria inhabiting surface waters of humic lakes are exposed to increased oxidative stress. In order to investigate the negative effects of light and photochemically generated ROS we monitored diurnal cycles of bacterial activity and growth in the humic south-west basin of Lake Grosse Fuchskuhle. High solar radiation caused strong inhibition of bacterial ^{14}C-leucine and ^{14}C-acetate uptake in surface waters and increased the fraction of membrane-damaged cells assessed by life/dead staining. The observed inhibition was paralleled by the formation of ROS as singlet oxygen and hydrogen peroxide. The impact of increased oxidative stress on bacterial community composition was assessed by PCR DGGE targeting 16S rRNA gene analysis using *Bacteria* and group-specific primers. In addition, bacteria were labeled with 5-Bromo-Uridine (BrdU) in water samples obtained throughout a diurnal cycle experiment. DNA labeled with BrdU was captured by monoclonal antibodies and PCR-DGGE pattern prior and after capture of BrdU labeled DNA were compared. These results also showed that several phylotypes were inhibited by high solar radiation and that cell division only occurred during hours of low solar radiation or during the night. Isolates representing predominant bacterial phylotypes were incubated in the surface water layer by using dialysis bags to verify our data. *Novosphingobium acidiphilum,* a persistent species of the south-west basin, was not hampered in assimilation of leucine and acetate by solar radiation. In contrast, leucine and acetate uptake by *Polynucleobacter necessarius* - a predominant *Betaproteobacteria* representative - was strongly inhibited during solar radiation exposure. In conclusion, species-specific patterns of metabolic activity occur in dominating bacterioplankton phylotypes, which are strongly related to solar radiation and subsequent photochemical reactions generating ROS.

MDP030

Microbial and Picoeukaryotic Community Composition of the Atlantic Ocean

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The Atlantic Ocean is a highly diverse body of water which Longhurst et al. (1995) first classified into distinct biogeographical provinces. Biogeographical provinces are categorized based on characteristic biogeochemical processes and on the biodiversity, specifically of primary producers. Marine microbial communities are known to be selected by and contribute to the specific biogeochemistry of their environments. They are the key organisms for the degradation of organic matter derived from primary producers but can also make up a significant fraction of the primary production. For example cyanobacteria such as Prochlorococcus can be responsible for > 40% of the primary production in both Atlantic gyres. In previous studies of the community composition using fluorescence in situ hybridization (FISH) it was shown that there is a change the horizontal distribution in the North and South Atlantic Ocean (Schattenhofer et al.,

2009). However these studies have only investigated the microbial community with a coarse taxonomic resolution.
During the AMT22 on RRS James Cook (Southampton, UK to Punta Arenas, Chile, 10th Oct to 24th Nov 2012) we examined the bacterioplanktonic and picoeukaryotic community composition in different size fractions along a transect from 50° N to 50° S. We applied in depth next generation sequencing for a comparative LSU and SSU rDNA analysis. The analysis of the V3 region of the 16S rDNA of Bacteria and Archaea and the D1/D2 region of the 23s rDNA of eukaryotic microalgae will allow for a comprehensive insight into microbial diversity patterns in the Atlantic Ocean. Due to the distinct difference in the biogeochemistry of the provinces we expect to see a transition in the dominant microorganism depending on the biogeochemistry of the province and the associated picoeukaryotic community.
This study will help us to understand the distribution of microbial communities along an environmental gradient and may highlight factors controlling the distribution of marine microorganisms.
The analysis of both the picoeukaryotic and microbial diversity will allow us to test for specific associations between distinct bacterioplanktonic and picoeukaryotic taxonomic clades.

Schattenhofer, M., Fuchs, B.M., Amann, R., Zubkov, M.V., Tarran, G.A., and Pernthaler, J. (2009) Latitudinal distribution of prokaryotic picoplankton populations in the Atlantic Ocean. Environmental Microbiology 11: 2078-2093.

MDP031

Formation of N-acetyl Sugars by Chitinases is the primary step of chitin hydrolysis by microorganisms in an agricultural soil

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Enzymatic cleavage of chitin can be conducted through hydrolysis of the β-1,4-bonds by chitinases or through prior deacetylation to chitosan and subsequent hydrolysis by chitosanases. In an aerated soil, O_2 distribution is heterogeneous and dynamic on the micro- to millimeter scale. Therefore, different redox processes, such as fermentation or O_2 respiration, can simultaneously be active on the degradation of chitin at the level of the system. The objective of the current study was (a) to evaluate the effect of O_2 availability on microbial processes and taxa involved in degradation of chitin, chitosan and its hydrolysis products *N,N´*-diacetylchitobiose ($GlcNAc_2$), N-acetylglucosamine (GlcNAc), and glucosamine (GlcN) and (b) to resolve the mechanism (direct vs. indirect) for chitin breakdown in an agricultural soil. A glycoside hydrolase (GH) family 18 chitinase gene library was dominated by genotypes that were novel. Known genotypes affiliated with those of *Beta-* and *Gammaproteobacteria, Verrucomicrobia, Acidobacteria, Actinobacteria, Firmicutes, Planctomycetes,* and *Eukaryota*. Supplemental chitin, $GlcNAc_2$, GlcNAc, and GlcN were mineralized to CO_2 under oxic conditions, and concentrations of NH_4^+ and NO_3^- increased, indicative of ammonification and nitrification. Under anoxic conditions, chitin, $GlcNAc_2$, GlcNAc, and GlcN were metabolized to CO_2, H_2, acetate, propionate, and butyrate. NO_3^- was completely consumed during the experiment. Anoxic soil slurries went black in treatments that were incubated for more than 20 days, suggesting the formation of ferrous iron. Thus, both NO_3^- and Fe^{3+} were apparently respired by soil microbes. TRFLP analysis revealed that different chitinase genotypes responded to supplemental chitin under oxic and anoxic conditions. In contrast to chitin, chitosan was not degraded within four weeks under oxic and anoxic conditions. The results suggest that (a) O_2 activated chitinolytic and saccharolytic aerobes, whereas anoxia activated chitinolytic and saccharolytic fermenters, NO_3^- respirers, and likely fatty acid-utilizing Fe^{3+} reducers, and (b) cleavage of β-1,4-bonds by exo- and endo-chitinases was the predominant mechanism of chitin breakdown and not deacetylation to chitosan.

MDP032

Distribution of `sodorifen´ emission throughout the genera Serratia

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The Gram-negative Enterobacteriacea Serratia sp. 4Rx13 emits a complex bouquet of volatile secondary metbolites including the unique major compound 'sodorifen'(octamethylbicyclo(3.2.1)octadiene) (1, 2). As the structure of 'sodorifen' is so splendorous, its biosynthesis is also completely unknown. Due to this unusualness we wanted to determine how widespread the 'sodorifen' emission is in the genus Serratia. The volatile emission of different Serratia species (S. proteamaculans, S. marcescens, S. odorifera, S. plymuthica, S. liquefaciens, S. rubidaea) were thoroughly analyzed by GC/MS technique and compared with the profile of S. odorifera 4Rx13. In total 98 different compounds were detected. Three ketones were commonly released by S. proteamaculans, S. marcescens, S. odorifera, S. plymuthica. However, 'sodorifen' was only emitted by three out of fifteen Serratia species/isolates. Interestingly, it could be shown, that only isolates of the clade S. plymuthica which were isolated from plants grown in Mecklenburg-Vorpommern (Germany) were able to produce this unusual and novel volatile. Future work will focus on the elucidation of the biosynthetic pathway and its origin.

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2 Von Reuss S., Kai M., Piechulla B., Francke W. (2010) Octamethylbicyclo(3.2.1)octadienes from Serratia odorifera. Angewandte Chemie 122: 2053-2054, 2010, Angewandte Chemie Int. Ed. 49: 2009-2010.

MDP033

Impact of Soil Properties on Communities of Nitrate Reducers and Denitrifiers

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Denitrifying prokaryotes are facultative aerobes that catalyse the reduction of nitrate and nitrite to nitrous oxide and molecular nitrogen. Soil denitrification is the main source but also a temporary sink of the greenhouse gas nitrous oxide and depends on the denitrifying community. The impact of soil properties and land-use on nitrate reducer and denitrifier communities was assessed. Six grassland and six forest soils under contrasting land-use type (grassland and forest) and intensity (managed and unmanaged) were studied with 454 pyrosequencing using nitrate reductase and N_2O reductase encoding genes (*narG* and *nosZ*, respectively). Soil pH ranged from 4 to 7.5 and water content from 13 % to 60 %. Nitrate concentration in soils varied from 0.4 to 2.8 μmol g_{DW}^{-1}, ammonium concentration from 15 to 25 μmol g_{DW}^{-1}, total N content from 1 to 21.5 mmol g_{DW}^{-1} and total C content from 0.14 to 0.2 mmol g_{DW}^{-1}. Soils of contrasting land-use type and intensity showed no explicit differences in their properties. 23400 *narG* sequences were grouped into 20 species level OTUs affiliating with Beta-, Gamma- and Deltaproteobacteria, Actinobacteria, Deinococci and Bacilli. 18010 *nosZ* sequences were grouped into into 22 species level OTUs affiliating with Alpha-, Beta- and Gammaproteobacteria. Canonical correspondence analysis revealed a high influence of land-use type and pH on *narG* containing communities. *NosZ* containing communities were influenced by land-use type and pH as well as ammonium concentration and water content. The impact of land-use type on denitrifiers was more evident on *nosZ* than *narG* communities. The combined data suggest that land-use type and pH shape nitrate reducer and denitrifier communities in soil while the latter are also affected by ammonium concentration and water content.

MDP034

Specific epibacterial communities on marine macroalgae: Distribution patterns of the Roseobacter-clade

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In marine ecosystems sessile eukaryotes like macroalgae represent ecological niches for diverse marine bacteria. By producing carbon compounds like extracellular polysaccharides, the algae provide special habitats with high bacterial species variability and strong competition for space and limiting resources (Long and Azam, 2001; Grossart et al., 2004). Members of the Roseobacter clade are widespread in the marine environment and can make up to 25% of the whole bacterial community (Wagner-Döbler and Biebl, 2006; Brinkhoff et al., 2008). Roseobacters were often found associated with marine eukaryotes like macroalgae (Wagner-Döbler and Biebl, 2006) however no systematic study was performed yet.
We sampled a variety of different species of marine macroalgae at the Spanish Atlantic coast of Galicia. Diversity of roseobacters on surfaces of brown, red and green algae was examined by a Roseobacter-clade specific 16S rRNA gene-based PCR-DGGE approach. On all investigated algae, roseobacters were found to be present. Furthermore, cluster analysis of the DGGE patterns revealed a highly diverse composition of roseobacter species. To further investigate the diversity of these bacteria on selected brown algae, a clone library was performed based on a Roseobacter-clade specific PCR and by 454 sequencing of 16S rRNA gene amplicons, previously amplified with eubacterial primers. The phylogenetic affiliation of the obtained sequences showed a broad distribution within the Roseobacter clade.
Previous studies revealed a promoted biosynthesis of antimicrobial compounds by the high level of competition on the algal surface for nutrients and space (Slattery et al., 2001; Grossart et al., 2004). Therefore interesting bacterial clusters were further analyzed with respect to their special interaction between the algal species and the colonizing bacteria. New strains isolated from the macroalgae were tested, e.g., for their ability to produce antimicrobial secondary metabolites.

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MDP035

Spatio-temporal dynamics of archaea in the rooted top soil of wetland rice

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The rooted top layer of paddy soils cultivated with wetland rice represents a highly dynamic environment in terms of biogeochemical and soil structural conditions. As a result of continuous flooding, anoxic conditions accompanied by low redox potentials prevail in the root-free bulk soil while oxic conditions occur in areas of oxygen availability, i.e. the rhizosphere and the soil surface layer. Especially oxygen-sensitive archaea such as methanogens are mainly affected by these biogeochemical dynamics. To study the related effects on the seasonal and spatial distribution of archaeal populations, rhizotron and microcosm experiments were conducted throughout an entire growing season of wetland rice.
Soil samples were taken from representative areas of the bulk soil, the oxidized layer, and the rhizosphere at main plant growth stages from the rhizotron boxes. In parallel, the soil texture effect was studied in microcosms taking soil samples within the top 5 cm before and after drainage towards the end of the vegetation period. Cell numbers of the domain *Archaea* and selected phyla were determined by catalyzed reporter deposition-fluorescence in situ hybridization (CARD-FISH). The structure and diversity of microbial communities was analyzed by polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) with sequencing of selected bands.
Seasonal effects were mainly visible in the rhizosphere, as microbial populations including methanogenic archaea showed highest cell numbers at flowering stage of rice plants. Members of *Methanocellales* were identified as predominant archaeal populations in all root-zone areas. In contrast to bacteria, the communities of archaea in the root-zone of a paddy soil seemed to be less altered by seasonal and spatial variations of biogeochemical conditions. The analysis of differently textured soils showed mainly changes in terms of population densities, which were significantly different when the dynamics of habitable pore space was taken into account.

MDP036

Bacterial communities in freshwater sponges

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Symbiotic associations between microorganisms and marine sponges have been shown to be interesting model systems for interaction studies between bacteria and metazoans. A most important feature is the exploitation of antimicrobial agents either produced by the sponge or by the sponge-inhabiting prokaryotes. However, microbial communities in freshwater sponges have been rarely addressed so far. Subjects of our studies were abundant sponge genera like *Ephydatia*. Here, we investigated the microbial community in the sponge tissue and putative mechanisms of vertical transfer of symbionts by resting stages (gemmulae). Though seemingly symbiont-free, a low number of prokaryotes remained attached to gemmulae. Other prokaryotic phylotypes were directly taken up by the sponge from the surrounding water and enriched in the establishing sponge biofilm. Denaturing gradient gel electrophoresis documented the dynamics of the community during resting stage formation. Several hitherto unknown bacteria, distantly related to various phylotypes identified in freshwater samples, were identified and/or isolated so far, including an oscillatorioid cyanobacterium and a *Polynucleobacter*-affiliated β-proteobacterium.

MDP037

Cryptogam covers on sepulchral monuments and re-colonization of a marble surface after cleaning

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Open-air sculptures exposed to weather and pollution are deteriorated by physical and chemical factors, but also microbial contamination is involved in biodeterioration. Different cleaning methods and conservation procedures were developed, but the re-colonization of freshly cleaned surfaces by aeroterrestrial microbial communities is up to now poorly understood. A comparative study addressing the composition of algal and fungal communities on a marble sculpture was conducted, based upon the analysis of 18S rRNA gene clone libraries from environmental samples. Overall 110 clones were retrieved from a blackish old grown biofilm cover and 112 clones from the same surface one year after cleaning treatment. The composition of the fungal community (12 OTUs) indicated significant differences between the old grown biofilm and the cleaned surface. While the former was dominated by the ascomycetes Rhinocladiella, Glyphium and Capnodiales, the black yeast Sarcinomyces was clearly dominant one year after cleaning, but could not be retrieved from the old grown black biofilm. The green algal community (10 OTUs) was dominated by different phylotypes of the lichen algae Trebouxia, as well as the cosmopolite green algae Apatococcus and Stichococcus. No essential differences in the green algal community before and after cleaning could be observed. Scanning electron microscopy revealed different attachment mechanisms for fungi on such substrates.

MDP038

Electron transport by filamentous *Desulfobulbaceae* in salt marsh sediment

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Centimeter long filamentous *Desulfobulbaceae* have recently been found to mediate long distance electron transport (LDET) in marine sediment (Pfeffer et al., 2012). The electron transport seemed restricted to longitudinal strings within periplasmic channels observed along the filament. LDET by these bacterial cables couples oxygen reduction at the sediment surface to the oxidation of sulfide more than 1 cm below the oxic zone (Nielsen et al.,

2010). Presently all published data on LDET by cable bacteria are derived from incubations of organic rich, sulphidic sediment from Aarhus Bay (Denmark).
In the present study, the occurrence of LDET and cable bacteria was investigated in intertidal, sandy salt marsh sediment (Little Sippewissett Salt Marsh, Massachusetts, USA). Sediment was defaunated by sieving and incubated with overlying aerated sea water. After five weeks, microsensor measurements revealed a >7 mm suboxic zone with no measureable oxygen and sulphide, and a pH peak in the oxic zone. These biogeochemical signatures clearly confirmed the development of LDET. Moreover, enrichment with filamentous *Desulfobulbaceae*, similar to the cable bacteria observed in Aarhus Bay sediment, was confirmed by fluorescence in situ hybridization (FISH) and sequence analysis. An additional experiment demonstrated that LDET also develops in salt marsh sediment with no overlaying water column. This observation makes the development of LDET and the presence of cable bacteria in intertidal and marsh areas plausible.

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MDP039

Characterization, abundance and distribution of the toluene-degrading Magnetospirillum sp. ANT6-6 strain isolated from the Planted Fixed-bed Reactor (PFR).

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Monoaromatic hydrocarbons of the BTEX group of compounds constitute one of the most common and serious threats to groundwater reservoirs, soils and indoor climates worldwide. Therefore, these compounds have attracted much attention regarding their biodegradation leading to the isolation of bacteria as well as the elucidation of biochemical pathways for their degradation under anaerobic conditions.
Previous molecular studies performed in Planted Fixed bed Reactor (PFR), wich is planted with the helophytes Juncus effusus and fed with mineral media containing toluene, elucidated that the catabolic activity (bssA gene) was 99% similar to Magnetospirillum sp. And also, it was detected 6% of the population is closely related to members of the genus Magnetospirillum involved in degradation of toluene.
An anaerobic cultivation screening of the PRF resulted in 13 cultures of bacteria with a spirillum shape. Phylogenetic analysis of 16S ribosomal DNA sequences and bcrA and bssA genes of strain ANT-6-06 revealed that this strain is most closely related to a Magnetospirillum sp. strain TS-6 previously characterized by Shinoda et al. (2005). It grew on toluene only under denitrifying conditions and other substrates, such as acetate, lactic acid and benzoate, supported aerobic growth. The strain exhibited optimal growth at pH 8.0 and grew on agar plates under aerobic conditions. Moreover, this study elucidated the quantification of the expression of these catabolic genes under different redox conditions in PRF.
Abundance and distribution of Magnetospirillum sp. ANT6-6 in the different compartments of the planted systems (pore water, roots and gravel) were examined using qPCR and Fluorescent In Situ Hybridization (FISH) in order to determine the contribution of these bacteria on toluene degradation in the PFR.

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MDP040

Fecal bacterial loads in a river as a function of climatic relevant factors: a modelling approach

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Fecal pollution of rivers bears a public health risk and is diminishing the quality of ecological services provided by these important ecosystems [1]. Because fecal bacterial survival and input is influenced by several environmental and climate sensitive factors [2] climate change may alter the hygienic situation of rivers in the future.
To get a deeper insight into the hygienic state of a river and its fecal pollution dynamics monitorings were conducted at subset rivers over a period of 12 month.
Abundances of *E. coli*, intestinal enterococci, and somatic coliphages were investigated together with a broad range of contextual parameters. Pairwise correlation analyses were carried out in order to identify environmental parameters that significantly influence fecal pollution in the river system.
Stepwise multiple linear regression models were developed for prediction of fecal indicator loads.
Results indicate that discharge and precipitation are the most influential factors, that affect fecal pollution. Furthermore it could be shown, that dry periods that preceded precipitation events contribute to high fecal indicator concentrations.
Multiple linear regression models provide a simple way to estimate bacterial loads on the basis of predictor factors and are an easy applicable tool for the quick use in water quality control. Those models can be used for example in risk assessment of recreational water use.

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MDP041

Bacteriocin production of staphylococcal nasal isolates

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Staphylococcus aureus is a major pathogen in hospital- and community-acquired infections. Colonisation of the anterior nares in about 30% of the population is a major risk factor for *S. aureus* infections. Recently the composition of the nasal flora has been investigated. Interestingly, the bacterial diversity in the human nose reaches from aerobic to strictly anaerobic bacteria. The most frequently occurring species are *Corynebacterium accolens/ C. macginleyi, S. epidermidis* and *Propionibacterium acnes*. In order to investigate if bacteriocin production might play a role during nasal colonisation, we analysed the bacteriocin production of nasal *Staphylococcus* strains.
The test-strains were casted in an agar plate and the nasal isolates were stamped on the plate. Various isolates showed growth inhibition zones of the test-strains. Transposon plasmids could be transformed into various strains and mutagenesis was performed.
Analysis of 93 stapylococcal nasal isolates offered that various strains produce bacteriocins against *Micrococcus luteus* and other nasal bacteria *(S. aureus, Corynebacteria, Moraxella, Propionibacteria…)*. The bacteriocin production of some nasal isolates turned out to be inducible by hydrogen peroxide or iron limitation.
One of these bacteriocins, produced by an *S. epidermidis* strain, could be characterized as a Nukacin-like lantibiotic with activity against *Micrococcos luteus, Moraxella catarrhalis, Streptococcus pyogenes and Corynebacterium pseudodiphtheriticum*.
Knowledge about the various interactions between staphylococcal and other nasal isolates could be important for effective *S. aureus* control strategies.

METV001

Towards a unified taxonomy for ribosomal RNA databases

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Taxonomic classification of rRNA sequences is one of the integral components of an rRNA database, alongside alignments. Whether a user is performing probe design for an environmental sample, or classifying thousands of unknown variable region tag sequences, taxonomy is under the spotlight. After all, the purpose of rRNA sequences, especially regarding *Bacteria* and *Archaea*, is the identification of organisms, and taxonomic classification identifies an unknown organism and attaches the previously described properties of a taxon. The three rRNA databases; RDP-II, Greengenes and SILVA, have different approaches to taxonomy curation. This creates a “richness” of taxa names, bringing along certain discrepancies between classifications. For example, The Bergey’s Outlines are followed by all three resources, despite some differences, but other non-validly

published taxa, *Candidatus* taxa, and environmental clades differ greatly from one resource to the other.
We believe that, from a taxonomy perspective, the three rRNA databases should aim at the very least for providing users with the "same" taxa names, regardless of the dataset and classification method used. Such reconciliation would depend on determining a reference set of sequences which share the same classification between three databases, and of course better sharing of data. Reconciliation of environmental clades (clades without described cultivated representatives), however, will require more effort. A possible roadmap may involve examination of the conventions used by three databases for environmental clades, and determination of stable phylogenetic clusters that all parties agree on. For these stable clusters, either taxon names that are already ascribed can be used, or new names based on an agreed convention can be devised. In order for these environmental taxon names to be persistent, it will also be desirable to build a registry, which can include data such as the original publication naming the clade, reference sequence for the clade, habitat patterns, or name changes to clusters.
In an effort to demonstrate the power and utility of taxonomic collaboration between databases, a new effort termed "Eukaryotic Taxonomy Working Group" has been initiated and has implemented a new hierarchical classification scheme for eukaryotic 18S rRNA sequences, which will be shared among all three resources.

METV002

Be aware of your nucleic acid extraction bias: insights from qPCR and deep sequencing of DNA and RNA extracts from soil

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Soil is one of the most complex habitats with a huge diversity of physicochemical properties. Modern sequencing technologies allow in depth analysis of total and active microbial communities based on analysis of DNA and RNA, respectively. However, the efficiencies of nucleic acid extraction methods vary substantially and introduce biases. Here, we compare the efficiencies of several commercial and laboratory-established methods for DNA/RNA extraction from three contrasting grassland soils by (i) quantification of total DNA and RNA, (ii) enumeration of 16S rRNA genes and transcripts using quantitative PCR, and (iii) pyrosequencing-based analysis of the bacterial community composition. Our results indicate that quality and yield of nucleic acids as well as abundance of 16S rRNA genes and transcripts differed considerably with respect to both the applied extraction method and the analyzed soil. Moreover, the relative abundances of dominant soil taxa varied by a factor of up to 10, based on a total of 481,572 16S rRNA gene sequences. The phospholipid fatty acid patterns revealed that Gram-positive bacteria were underrepresented in all DNA extracts, probably due to insufficient cell lysis. Our results demonstrate that the apparent composition of both the total and the active bacterial community in soils highly depends on the nucleic acid extraction method, indicating different lysis efficiencies for different community members. Some phyla (e. g., *Proteobacteria*) seem to be more affected than others (e. g., *Acidobacteria*).
We therefore conclude that, despite the undeniable advantages of method standardization in general, the trustability on a single standardized protocol for nucleic acid extraction suitable for a large range of soils, target organisms and application purposes belongs to wishful thinking. At best, standardization would only be possible within less broad applications.

METV003

A novel detection method for visualizing environmental microbes with low rRNA content and low cell permeability by using *in situ* HCR-FISH

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Catalyzed reporter deposition-fluorescence *in situ* hybridization (CARD-FISH) has been widely used for detecting environmental microbes with low rRNA content. However, CARD-FISH requires high-molecular-weight enzyme-labeled probes for signal enhancement, and it is difficult for such probes to penetrate the target cells. Moreover, a universal cell wall treatment method for all cell-wall types has not yet been reported. In this study, we developed a novel sensitive FISH for improving probe permeability by using in situ hybridization chain reaction (HCR)-FISH. The HCR is used to detect targeted single-stranded DNA by extending 2-hairpin oligonucleotide DNA probes. To detect microbes, *in situ* HCR-FISH uses a connector probe, which has the regions of the target sequence of the rRNA and origination sequence of HCR extension for detection, along with fluorescently labeled 2-hairpin oligonucleotide probes that extend from the connector probe.
The signal amplification by *in situ* HCR-FISH was checked using *Escherichia coli*. The signal intensity of *in situ* HCR-FISH was found to be stronger than that of standard FISH. The cell permeability of *in situ* HCR-FISH was next evaluated using *Methanosaeta concilii*, which requires a cell-wall treatment for CARD-FISH. *M. concilii* cells were detected without cell-wall treatment by *in situ* HCR-FISH. This result showed that the probe permeability of *in situ* HCR-FISH is greater than that of CARD-FISH. Finally, *in situ* HCR-FISH was utilized for detecting marine bacteria as environmental microbes with low rRNA content. Detection rates of *in situ* HCR-FISH and CARD-FISH were similar after cell-wall treatment. However, the detection rate of *in situ* HCR-FISH without cell-wall treatment was higher than that of CARD-FISH. These results indicated that *in situ* HCR-FISH has a potential to be utilized as a sensitive FISH and is invaluable in detecting microbes with low cell permeability.
To summarize, our study is likely the first to report on the use of *in situ* HCR-FISH for detecting environmental microbes. In addition, the combination of *in situ* HCR-FISH with single cell analysis becomes a powerful tool for revealing the metabolism of uncultured microorganisms.

METV004

Evidence for heterotrophic microorganisms in diffuse fluids at two hydrothermal systems

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Short chain fatty acids such as formate and acetate have been repeatedly found in rock-hosted hydrothermal fluids. Modelling shows that formate and acetate could occur in nM to μM concentrations in fluids from basalt-hosted systems. Although mixo- and heterotrophic microorganisms have been isolated from active vent sites, little is known about their *in situ* activity, identity and abundance in hydrothermal fluids.
We studied the community composition and acetate utilizing activity of microorganisms in diffuse fluids from two hydrothermal systems: the basalt-hosted Menez Gwen (MG) system and the felsic hydrothermal field of the Manus Basin (MB). 16S rRNA gene diversity analysis and fluorescence *in situ* hybridization (FISH) were performed in fluids ranging from 4° to 72°C. To detect the general metabolic activity on the cellular level, fluids were short-term incubated with ^{13}C-acetate and ^{15}N-ammonium for subsequent analysis of >300 single cells by nanometer scale secondary ion mass spectrometry (nanoSIMS).

A newly designed FISH-probe, specific to *Nautiliales*-related epsilonproteobacteria, showed that these accounted for 90% of all acetate- and ammonia-consuming bacteria after 8h of incubation. Their absence in the plume and their high activity at 55°C indicated that this group could be important acetate-oxidizing organisms in the hydrothermal subsurface. In the fluids of the MB however, gammaproteobacteria constituted the largest fraction of ^{13}C-acetate- and ^{15}N-ammonia-consuming bacteria at 4° and 37°, whereas only very few epsilonproteobacteria were found to be enriched in ^{13}C. 16S rRNA gene analysis revealed organisms rather typical for pelagic sea water such as *Marinobacter* and *Alteromonas*.
Our results suggest the potential of heterotrophy and possibly also of mixotrophy by gamma- and epsilonproteobacteria in diffuse fluids and in the subsurface of different rock-hosted hydrothermal systems.

METV005

Compost gourmets - Using stable isotope probing for elucidating the carbon flux of the PAH degradation in composting environments and the responsible micro-organisms

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Polycyclic aromatic hydrocarbons (PAH) are toxic pollutants ubiquitous in the environment due to natural and anthropogenic processes, e.g. volcanic eruptions, incomplete combustion and spills of tar oil products. Therefore, intensive research is needed to optimize the clean-up of PAH contaminated sites. One approach is to add compost to PAH-containing soil in order to stimulate the native microorganisms able to use the pollutants as substrate for growth. However, the microbial key players in the compost-aided processes are unknown yet. This work aims to trace the fate of PAH derived carbon in soil-compost microcosms using ^{13}C-pyrene as a model PAH and to identify the key microbes responsible for the pyrene degradation by analyzing biomarkers. For setting up the experiment, triplicates of uncontaminated soil (Haplic Chernozem) were mixed with commercially available matured compost and supplemented with 100 mg kg^{-1} $^{13}C_6$-pyrene or for control with ^{12}C-pyrene or without pyrene. Seven samples were taken from the ^{13}C-microcosms and the controls during the incubation period of half a year. The pyrene degradation was determined by analyzing soil-compost extracts using a GC-MS, the mineralization was quantified by trapping the released CO_2 and $^{13}CO_2$. In order to check the label incorporation into the biomass, ^{13}C-lipids were targeted (PLFA-SIP), which at the same time allows a rough taxonomic estimation of the involved pyrene degrading organisms. ^{13}C incorporation into proteins was determined to identify active key microbes and functions by protein-SIP. Combining all the biological and physicochemical information from this experiment, we will gain better understanding of the carbon flux in composting environments and the responsible PAH degrading community, underlining the valuable role of compost in soil bioremediation.

METV006

The response of *Polynucleobacter necessarius* to light in a humic lake

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Epilimnic bacterioplankton of humic Lake Grosse Fuchskuhle is often dominated by *Polynucleobacter necessarius*, an ubiquitous ultramicrobacterium of the *Burkholderiales* (*Betaproteobacteria*). Hence cultured representatives of this species provide useful microbial model systems for investigating the cellular response to diurnal changes in environmental factors, e.g. light, nutrients and oxidative stress. In the SW basin of the artificially divided Lake Grosse Fuchskuhle, epilimnic bacteria were strongly inhibited by sunlight exposure. Either light or light mediated photochemical reactions may cause this effect leading to a decreased uptake of radiolabelled substrates, a decreased bacterial DNA synthesis and an increased fraction of membrane damaged cells. Bacteria were able to recover from the sunlight mediated inhibition. Subsequently, we assessed the underlying molecular responses in a cultured representative of *P. necessarius*. Dialysis bag cultures of this strain were grown in the surface water layer of the SW basin. Cultures exposed to sunlight and cultures covered with black plastic foil were sampled at 2 pm on a day with high solar radiation. Additional cultures were harvested before sunrise at 4 am after a period of ca. 6 hours without sunlight. In sunlight exposed cultures Illumina sequencing of total RNA extracts revealed a strong decrease in mRNA levels of genes encoding for central cellular functions such as energy metabolism (e.g. citric acid cycle genes), anabolic processes (e.g. synthesis of amino acids) and genes involved in cell division. In contrast, genes encoding oxidative stress response mechanisms (e.g. peroxiredoxin) were increased. Two dimensional gel electrophoresis of proteins from laboratory *P. necessarius* culture experiments revealed that light and oxidative stress caused a global proteome changes. In summary, our data show that *P. necessarius* strongly respond to sunlight exposure. Molecular mechanisms were triggered to overcome the effects of sunlight exposure or light mediated photochemical reactions, which are in turn prerequisite for *Polynucleobacter* to survive in the upper, sunlit water layer of freshwater ecosystems.

METV007

Microbes and root-soil interfaces: *in situ* analysis of single cells in rhizosphere research

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The understanding of soil-microbe-plant interactions is of increasing importance in rhizosphere research. Although influences of the plant host on microbial populations (and vice versa) are likely to be greatest in close vicinity to the root surface, the *in situ* analysis of microbial rhizoplane communities has been rarely conducted due to methodological limitations. Here, we present the application of fluorescence *in situ* hybridization with catalyzed reporter deposition (CARD-FISH) for a routine *in situ* identification, localization, visualization, and quantification of single microbial cells (i) on the root surface and (ii) in the associated rhizosphere soil. With emphasis on microbial rhizoplane communities, the spatial distribution of archaea, bacteria, and relevant microbial populations within these domains such as methanogens and methanotrophs were studied in the root-zone of a paddy soil. For the first time, quantitative data of single microbial cells colonizing the rhizoplane of soil-borne roots were obtained for an entire growing season of wetland rice. Comparable to the absolute abundance of archaea and bacteria in the rhizosphere soil, the cell numbers of rhizoplane communities increased towards the flowering stage of rice plant development. Archaea and bacteria were found to account for 13.2 and 62.6 $\times 10^3$ cells per rhizoplane surface (mm^2), respectively, while the numerical ratio of archaea:bacteria shifted towards the latter. Furthermore, spatiotemporal dynamics of archaeal and bacterial colonization patterns were monitored during the entire growing season, starting from patchy microbial clusters along axial grooves towards an almost uniform distribution of single cells on the rhizoplane. Areas of pronounced iron-deposition such as the root tip as well as lateral roots were heavily colonized by members of Betaproteobacteria, indicating a biotic formation of Fe-plaques on rice roots. The presented approach for the *in situ* detection and enumeration of single microbial cells in the rhizosphere may close the gap between the often separately studied soil and plant compartments. Furthermore, the application of recent methodological developments such as gold-FISH will be discussed towards a better understanding of the mechanisms behind soil-microbe-plant interactions.

METV008

Survival of human adenoviruses, noroviruses, indicator bacteriophages and bacteria in sewage-contaminated streaming waters: Assessing the impact of environmental factors

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In Germany, raw water quality assessments are still based on bacteriological indicators but there is increasing demand for using additional indicators for fecal contaminations as well as reference organisms to reflect the behavior

of other pathogens, like viruses and parasites. The suitability of surrogate model viruses, their role for improving concepts in drinking water and bathing water managements and their request to be representative for specific human viruses or groups of certain virus families is still challenging, especially under rapidly changing environmental conditions. Therefore, new insights into virus survival rates under various environmental conditions are important for the assessment of natural and technical processes of virus removal from sewage-contaminated waters. The impacts of environmental conditions on monitoring results and correlation variances between viruses and surrogates were determined under well-controlled conditions of an outdoor technical-scale facility, representative for rivers and other streaming waters. Two outdoor channels, each containing 30.000 liters of streaming water were spiked with 1-5% of fresh primary waste water effluent as a natural source of microorganisms and viruses. Special attention was given to the ambient temperature and the effect of sunlight and its UV components on the survival of bacteria and viruses. Open and covered channels of streaming water were monitored in parallel throughout different seasons. Compared to those of bacteria, virus declines were significantly prolonged. During these experiments, the levels of indicator bacteria did not correlate with those of bacteriophages and viruses. Over a wide range of concentrations, somatic coliphages displayed similar inactivation characteristics as human pathogenic viruses, like adenoviruses and noroviruses. Supplementing the use of bacterial parameters by viral indicators of human fecal pollution may be useful for risk assessments and a helpful step to reduce the need for direct testing of human viruses in contaminated waters.

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METP001

Soil and land use type affect the structure and function of bacterial community in subtropical savannah soils

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Drylands (i.e., arid, semiarid, and subhumid areas) cover approximately 40% of the continents and currently extend due to climate change. However, more than one billion people depend on agriculture in these regions. Besides water supply, nutrients like ammonium, nitrate and phosphate limit plant production in these areas. Since nutrient cycling and mobilization are mainly driven by bacteria and their metabolic activity the interdependencies between the structure and function of bacterial communities and the biogeochemical processes were examined. Sampling sites are located in nutrient limited semiarid soils of North-Eastern Namibia and differ with respect to soil type (sand, i.e., Kalahari sands, and loamy sand, i.e., old flood plain soils) and land use (fallow, drought and irrigation agriculture, bushland, and riparian woodland). Illumina-based high throughput sequencing analysis of partial 16S rRNA genes revealed that the soil type rather than the land use type determines the composition of the active bacterial community. Bacterial activity was assessed by the determination of exoenzyme kinetics and nitrogen turnover rates. Aminopeptidase, β-glucosidase, β-xylosidase, and phosphatase involved in the breakdown of oraganic matter were examined with fluorescently labeled substrate analogues. Exoenzyme activities were higher in unmanaged loamy soils than in anthropogenically influenced soils. Interestingly, results indicate that carbon turnover was faster in loamy than in sandy soils. The pool dilution technique was used to quantify ammonification and nitrification rates. Ammonification and nitrification were strongly affected by the land use type and reached the highest values in woodland soils and lowest values in bushland and agricultural soils. Based on the presented results, we conclude that microbial activities in semiarid savannah soils decrease with increasing anthropogenic impact.

METP002

Biosynthesis of lysolipin: microbial communication or active defense?

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Streptomyces tendae Tü 4042 produces the aromatic polyketide antibiotic lysolipin (1). Lysolipin is highly active against Gram-positive bacteria (MIC < 50 nM) and targets probably a yet unidentified component in the bacterial cell envelope (3). It also shows strong cytotoxic effects on eucaryotic cells and might therefore be involved in active defense against bacteria as well as higher organisms.

The lysolipin biosynthetic gene cluster was identified, sequenced and annotated. It comprises 44 genes putatively involved in biosynthesis, regulation and export of the antibiotic. All genes necessary for lysolipin production are located on a 42 kb cosmid as shown by successful expression of lysolipin in the heterologous host *Streptomyces albus* (2).

Its carbon-skeleton is built in an iterative process by a type II polyketide synthase consisting of a ketosynthase α (KS α, LlpF), a ketosynthase β (KS β, LlpE) and an acyl carrier protein (ACP, LlpD) condensing malonate units. Backbone formation is followed by tailoring steps like oxygenations, methylations or halogenations.

To elucidate whether lysolipin is used for active defense or microbial communtication it is necessary to identify signals triggering the lysolipin production as well as its regulation. Furthermore the molecular target and the mode of action of the antibiotic has to be identified.

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METP003

Quantification of carbon fixation in phylogenetically defined bacterial populations from marine sediments by FISH, flow sorting and scintillation counting

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The quantification of carbon assimilation by specific populations of uncultured microorganisms mostly relies on fluorescence *in situ* hybridization combined with semi-quantitative, isotopic-tracer approaches like microautoradiography (MAR-FISH) or on methods with a low sample-throughput like nanoSIMS. Flow cytometric cell sorting was previously used to measure the assimilation of radioactive substrates in individual populations that were distinguishable based on nucleic acid or protein content (1). In our study, we flow-sorted individual bacterial populations from marine sediments after FISH to measure assimilation of radio-labelled (^{14}C) bicarbonate in the sorted cell fractions. As controls fluorescent beads and *E. coli* cells were flow-sorted to determine background radioactivity. By scintillography of 50,000 sorted cells per population we could exactly quantify the mean $^{14}CO_2$ fixation in *Bacteria*, *Gammaproteobacteria* and in the uncultured WS-Gam209 group that is related to gammaproteobacterial sulfur oxidizers (2). Despite only contributing 20-30% to total cell counts *Gammaproteobacteria* accounted for 68-79% of the total $^{14}CO_2$ fixation. After determining the relative abundance of assimilating WS-Gam209 cells by MAR-FISH (2) we calculated a mean cell-specific fixation rate of 7.5 fg C day^{-1}, which is highly consistent with rates measured for uncultured green-sulfur bacteria by nanoSIMS (3). In support of a lithotrophic metabolism of the WS-Gam209 group we detected key genes for sulfur- (*apr*A*)* and hydrogen- (*hup*L) oxidation in genomic DNA amplified from a single cell. Consistent with their heterotrophic lifestyle little carbon assimilation was measured in sorted cells of the alphaproteobacterial *Roseobacter*-lineage.

The combination of FISH, flow sorting and scintillography allows a high sample throughput (several 100,000 cells per day), an exact quantification of bulk assimilation in defined populations and can be easily combined with single-cell approaches such as microautoradiography and nanoSIMS. With this method in hands, we are now able to unravel the total and the relative contribution of individual microbial populations to carbon assimilation, which gives deeper insight into the functionality of microbial communities in marine sediments.

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METP004

First come first served: elucidating the microbial resuscitation cascade in biological soil crusts

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Biological soil crusts (biocrusts) are photosynthetic mats formed through an association of prokaryotic and eukaryotic microorganisms with soil particles; they are found in terrestrial ecosystems where vascular plant coverage is limited by abiotic conditions, such as drylands. As the largest

terrestrial biome, drylands cover around 40% of the land surface and are therefore the primary habitat for most biocrusts. In these ecosystems water availability is limiting and the crusts are dormant most of the year. Short and sporadic pulses of rain lead to a burst of microbial activity in the crusts followed by a return to dormancy upon dry out. A large body of literature exists on bioctusts going back at least to the beginning of the 20th century, classifying its various forms, habitats, compositions and functions. Yet while the photosynthetic and fungal members of these lifeforms received much attention, all other bacterial and archaeal inhabitants of the crust were only sporadically mentioned and have rarely been the focus of research. In this work we set out to study the dynamics of the active bacterial community in two biocrusts from an arid and a hyperarid region in the Negev Desert, Israel, under light-oxic and dark-anoxic incubation conditions after simulated rainfall. We used $H_2{}^{18}O$ for hydrating the soils and analysed the bacterial community using an RNA-Stable Isotope Probing approach coupled with 454-pyrosequencing. Our experimental set-up allowed us to reliably mimic the unique conditions distinguishing the top and lower part of a biocrust. Two distinct bacterial communities developed under each incubation condition. The active anaerobic communities were initially dominated by members of the order Bacillales which were later replaced by Clostridiales. The aerobic communities on the other hand were dominated by Sphingobacteriales and several Alphaproteobacteria (Rhizobiales, Rhodobacterales, Rhodospirillales and Rubrobacteriales). Actinomycetales were the dominant bacterial order in the dry soils but quickly collapsed and accounted for less than 1% by the end of the incubation. Our study shows that biocrusts host a diverse community whose members display complex interactions as they resuscitate from dormancy.

METP005

Screening for degraders: novel identification approach using substrate-specific radiolabeling of cells isolated in microcompartments

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The dominant drivers of organic compound turnover in environmental systems are prokaryotes. Although the general catabolic mechanisms are known for many compounds, in the majority of habitats the particular microbes responsible for the *in situ* turnover are difficult to identify. Currently, this identification of relevant microbial key players is a very time consuming or even futile endeavour due to environmental complexities and heterogeneity of microbial communities. It often fails due to a high number of non-cultivable and unknown microbes, issues that cannot be addressed by standard means such as laboratory cultivation or detection of functional genes for instance via hybridization probes or sequencing.
We are pursuing a novel screening approach for microbes with *in situ* activity that seeks to overcome current limitations by independence from cultivation and prior phylogenetic or catabolic information. Here we show the first results of the development of a Radio Isotope Probing method, which is based on the utilization of ^{14}C labelled compounds. It encompasses a short incubation of an environmental sample with a ^{14}C labelled substrate followed by encapsulation of cells with subsequent microautoradiography (MAR). Within these microcapsules compound-transforming microbes can be spatially isolated, separated based on visual differences caused by MAR, and investigated via nucleic acid analyses. The main principle has been proven with a comparatively large capsule scale. Current optimisations target process automation and minimisation down to the single cell-level.

METP006

Analysis of the carbon and nitrogen metabolism of nitrifiers using protein-based stable isotope probing (Protein-SIP)

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Nitrification is not only an important ecosystem process in the global nitrogen cycle, but also plays a key role in nitrogen removal from wastewater. The identification of nitrifiers and the investigation of their metabolic functions can help to understand their diversity, distribution, and activity in different ecosystems. However, due to the generally low growth rates of nitrifiers and the limited numbers of readily characterized phenotypes, the links between species and functional diversity of nitrifiers are generally not well understood. Stable isotope probing (SIP) based methods may help to identify key nitrifiers in distinct ecosystems. Here we set up a method for detecting the assimilation of isotopically labeled ammonium and carbon dioxide by nitrifiers using protein-based stable isotope labeling (Protein-SIP). Two model strains, *Nitrobacter winogradskyi* ATCC 25978 (oxidizes ammonium to nitrite) and *Nitrosomonas europaea* ATCC 25391 (oxides nitrite to nitrate), were cultivated on $^{13}CO_2$, $^{15}NH_4$ (*N. europaea*) or $^{15}NO_2$ (*N. winogradskyi*). Protein samples were collected at different incubation times and analyzed by global proteomic approach. 702 proteins from ^{13}C-labeled and 699 proteins from ^{15}N-labeled *N. europaea* culture were identified LC-MS/MS analysis. 445 proteins from ^{13}C-labeled and 339 proteins from ^{15}N-labeled *N. winogradskyi* cultures were identified. These proteins mainly consisted of transporter, regulatory and signaling proteins as well as those involved in ammonium oxidization (*N. europaea*), nitrite oxidation (*N. winogradskyi*), and biosynthesis. *N. europaea* oxidized approximately 7.1 mM ammonium within 24 h cultivation; identified proteins showed up to 87 atom% ^{13}C and 95 atom% ^{15}N incorporation, with ^{13}C and ^{15}N labeling ratios of 0.75 and 0.76. *N. winogradskyi* oxidized approximately 14.5 mM nitrite within 24 h cultivation; identified proteins showed 92 atom% ^{13}C and 96 atom% ^{15}N incorporation with labeling ratios of 0.9 (^{13}C) and 0.8 (^{15}N).
Proteins involved in the nitrification process showed high ^{13}C- and ^{15}N-labeling ratios, demonstrating that protein-SIP with $^{13}CO_2$ or $^{15}NH_4$ has a potential to detect specific active nitrifiers and their metabolic functions in nitrifying microbial communities.

METP007

Assessment of inhibitory effects during qPCR analyses of environmental samples and elimination of inhibitors during sample preparation.

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Detection of viruses in surface waters is often linked to the concentration of large sample volumes and constrained by inhibitory substances interfering with nucleic acid extraction or methods of detection. Inhibition has strong influence on the results of a measurement as well as on the interpretation of the data. Therefore, monitoring of inhibitory effects is greatly recommended in order to assess the reliability of results. Besides the monitoring, inhibitory substances can be eliminated during the process of sample preparation. However, each step may be attended by yield loss. Elimination procedures should therefore be studied in connection with the specific detection system used, so that the benefit of inhibitor elimination can be balanced against the risk of target loss and in order to interpret collected sample data in a reasonable way. For monitoring of virus concentrations in contaminated water samples, our group concentrates sample volumes of 10 L to volumes of 100 μl by glass wool filtration, beef extract flocculation and nucleic acid extraction. Viruses are subsequently detected by quantitative Real Time-PCR (qPCR). Co-concentration of inhibitory substances during the concentration process is inevitable. Here, we present a control system to monitor inhibition within PCR samples. It is applied routinely in our laboratory and is independent from the type of sample used. Using humic acids as a representative example of inhibitory substance and human adenoviruses as test viruses, we evaluated two promising methods for the elimination of PCR inhibitors. Our results indicate, that the majority of samples concentrated from environmental surface waters revealed inhibitory effects when applied to the PCR without prior purification or dilution. Purification by either of the presented inhibitor elimination methods reduced inhibition of humic acids by at least one $\log_{10}$ step. Concentration steps, although inevitable for many procedures, are the most critical steps in monitoring of environmental samples. Applying the inhibitor control system and elimination of inhibitors significantly improved the reliability of results of evironmental samples with large volumes.
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METP008

Systematic improvements of the GeneFISH Method for microbial gene detection

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GeneFISH is a method based on Fluorescence *In Situ* Hybridization (FISH). It simultaneously detects cell identity and gene presence in environmental microbes. The microbial identification is achieved by CARD-FISH, using oligonucleotide probes that target the 16S rRNA and a catalyzed reporter deposition (CARD) step to enhance the signal. Gene detection is performed using polynucleotide probes multiple times labeled with digoxigenin and a combined antibody-CARD signal amplification system. So far, a detection efficiency of <50% for low copy number of target genes limited the quantitative applications of the method. Furthermore, the long gene hybridization incubation (17 to 24 hours) made the protocol slow. Additionally, long period of incubations can increase the DNA and cell damage, decreasing the detection efficiency. The aim of this study was to systematically improve the geneFISH method in terms of detection efficiency, signal localization and time required.
For this purpose, we used *Escherichia coli* clones with different copy numbers of a target gene on plasmids (*unk* gene). For negative control we used another *E. coli* clone that lacked the target gene. To improve the detection efficiency, we increased the number of polynucleotide probes (300 bps) from one to twelve, by targeting consecutive locations on the same gene. For low (3-8) gene copies per cells, the detection efficiency ranged from 70% for one probe to 98% for 12 probes, with a plateau at ~93% starting with 4 probes. For high copy number (>100 copies), the detection efficiency was >90% for one probe. To increase the localization of the gene signal we tested different dextran sulfate (DS) concentrations (10%. 20%, 30% and 40%) and two tyramide concentrations (1 and 2 μg/ml) in the gene CARD step. The signal localization and detection efficiency was optimal using 2 mg/ml tyramides and between 20% and 30% DS. To decrease the time of experimentation, we tested different DS concentration in the gene hybridization buffer (10%, 15%, 20%, and 25%). The optimal concentration was 20%, which allowed a decrease of the gene hybridization time from 17 to 2 hours and of the minimum probe number from 4 to 3, while maintaining a gene detection efficiency of 95 %.
Our results indicate that the application of multiple probes increases the gene detection efficiency, enabling the application of geneFISH in a quantitative manner. Furthermore, doubling the dextran sulfate in the hybridization buffer significantly decreased the hybridization time.

METP009

A PCR-based microbial assay for the detection of anaerobic naphthalene degradation

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants listed by both the US-EPA and International Agency for Research on Cancer (IARC) as possible human carcinogens. The high molecular weight, limited water solubility, and strong adsorption to sediment matrices contribute to slow rates of natural attenuation and long-term environmental persistence, especially under anoxic conditions. The underlying need to efficiently assess PAH biodegradation potential in contaminated environments led to the present study.
Recently, two enzymes catalyzing key dearomatization reactions during anaerobic naphthalene biodegradation were identified in the sulfate-reducing enrichment culture N47 (Eberlein *et al*. accepted). The first enzyme catalyzes the ATP-independent reduction of 2-naphthoyl-CoA (NCoA) to 5,6,7,8-tetrahydro-2-naphthoyl-CoA (THNCoA). The second enzyme catalyses the ATP-dependent reduction of THNCoA to hexahydro-2-naphthoyl-CoA isomer (HHNCoA) and is extremely oxygen sensitive, in contrast to the first. DNA-based primers were designed to amplify specific regions of the NCoA reductase gene. These primers were used to create a clone library from one naphthalene-degrading strain (NaphS2, Galushko *et al*. 1999), N47 (Meckenstock *et al*. 2000), and two environmental enrichments from PAH contaminated sites in Germany and the Czech Republic, respectively.
Results led to the development of a degenerative primer set for amplification of a 300bp fragment. Positive results were obtained for NaphS2, NaphS3 and NaphS6 (Musat *et al*. 2009), N47, and three naphthalene degrading environmental enrichments.

Citations:
Galushko, A, Minz, D, Schink, B, and Widdel, F (1999) Anaerobic degradation of naphthalene by a pure culture of a novel type of marine sulphate-reducing bacterium. *Environ Microbiol* **1**:415-420.
Meckenstock RU, Annweiler E, Michaelis W, Richnow H-H & Schink B (2000) Anaerobic naphthalene degradation by a sulfate-reducing enrichment culture. *Applied and Environ Microbiol* **66**:2743-2747.
Musat F, Galushko A, Jacob J, *et al*. (2009) Anaerobic degradation of naphthalene and 2-methylnaphthalene by strains of marine sulfate-reducing bacteria. *Environ Microbiol* **11**:209-219.

METP010

Autecology of marine hydrocarbon-degrading sulfate-reducing bacteria based on methyl-alkyl succinate synthase (MasD) genes

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Marine gas and oil seeps are hot spots of microbial sulfate reduction because of a long term supply of hydrocarbons to natural microbial communities. Sulfate-reducing bacteria (SRB) of the diverse deltaproteobacterial *Desulfosarcina/Desulfococcus* (DSS) group have been shown to be key players in seep sediments and to be capable of alkane degradation [1, 2]. While long-chain alkane-degraders are subject of intensive research and several cultivated organisms are available, knowledge about *in situ* abundant and active short-chain alkane-degrading SRB is limited and only few isolates have been obtained so far [3].
In this study, we elucidate the diversity and autecology of short-chain alkane-degrading SRB at marine hydrocarbon seeps based on their methyl-alkyl succinate synthase genes (*masD)* encoding the activating enzyme in anaerobic alkane degradation. By the analysis of diversity and *in situ* quantification of the *masD*-carrying microbial community we aim to contribute to a better understanding of *in situ* hydrocarbon turnover and a possible response of the environment to oil spills.
The *masD* gene diversity was studied in 13 hydrocarbon seep sediments by parallel tagged sequencing on the 454-sequencing platform. The sediments differ e.g. in temperature, water depth, methane flux, or hydrocarbon composition. Results will be discussed with respect to diversity, biogeography and possible correlations with environmental factors influencing the short-chain alkane-degrading microbial community.
In parallel, a geneFISH detection system was developed for the *in situ* detection of *masD*-carrying microorganisms. Using geneFISH, *masD* gene presence is linked with cell identity in environmental microorganisms. First promising results were obtained in butane-degrading enrichment cultures from Guaymas Basin sediments.
The next step will be the application of our detection system on sediment samples from Guaymas Basin and afterwards, if successful, on other hydrocarbon seep sediments.

1 Kleindienst *et al*., 2012. Environ Microbiol. 14: 2689-710.
2 Widdel *et al*., 2010. In: Handbook of hydrocarbon and lipid microbiology. Springer, Berlin, Heidelberg: 1997-2021.
3 Kniemeyer *et al*., 2007. Nature 449: 898-901.

METP011

Characterization of potentially pathogenic *Vibrio* spp. by *rpoB*-DHPLC

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Question: Rising sea water temperature due to global warming enhances the conditions for *Vibrio* spp. to grow and disperse even in temperate waters of the North and Baltic Sea. Because of the increased incidence of Vibrio infections in the last years, a rapid and accurate method is required to analyze and identify complex *Vibrio* spp. populations, specifically potential pathogenic Vibrio species, in environmental samples. A PCR-DHPLC (Denaturing High Performance Liquid Chromatography) has been developed based on the *rpoB* gene of the genus Vibrio, which is a promising method to not only identify but also separate *Vibrio* spp. in mixed samples due to the different running behavior of amplified PCR products.
Methods: To facilitate the identification of potential human-pathogenic species we designed Vibrio specific primers based on *rpoB* sequences of *Vibrio* spp. strains isolated at Helgoland Roads (North Sea). These primers were combined to amplify fragments of ~500 bp of this *rpoB* gene. Using

the PCR products of four different Vibrio species, we systematically improved the DHPLC conditions, including column temperature and acetonitrile gradient. Finally, we compared the PCR-fragment separation with and without a 40-bp clamp attached to the amplification primers.
Results/Conclusion: We developed five primer-sets for different regions of the targeted *rpoB* gene and verified the primer-sets by successfully amplification of 20-23 different Vibrio species from a total number of 31. We could show that for optimal separation of the amplified fragments by DHPLC the column temperature plays a crucial role and needs to be adapted for each PCR-fragment individually, either with or without GC-clamp. The attached GC-clamp was essential for partial denaturing of all fragments at the same temperature, but generated ambiguous retention peaks. We will illustrate the development of this method in detail.

METP012
Transcriptional activation of hydroxylase-encoding structural genes associated with 2,4-DCP degradation in soil is mediated by earthworms

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Earthworms stimulate removal of certain xenobiotics in soil. 2,4-dichlorophenol (2,4-DCP) represents the initial degradation product of the herbicide 2,4-dichlorophenoxyacetic acid and is a component of wood preservatives. Degradation is initiated by a *tfdB*-encoded 2,4-DCP hydroxylase or by dechlorination and subsequent action of a *pheA*-encoded phenol hydroxylase. Degradation of 2,4-DCP occurs in soil and in the drilosphere, i.e., earthworm gut content, cast, and burrows. Earthworms (*Aporrectodea caliginosa*) accelerated the disappearance of 2,4-DCP in soil columns supplemented with 2,4-DCP relative to controls without worms. Most probable numbers (MPNs) of 2,4-DCP degraders after incubation in soil columns (a) in bulk soil of columns with and without earthworms approximated 6 x 10^5 and 6x 10^3 g_{DW}^{-1}, respectively, and (b) in burrow walls approximated 9 x 10^6 g_{DW}^{-1}. Earthworms and 2,4-DCP treatment stimulated *tfdB* and *pheA* gene copy numbers and transcription of *pheA*. 20,000 *tfdB* amplicons were obtained by barcoded amplicon pyrosequencing and were grouped into 58 OTUs based on a gene similarity of 80 %. Major OTUs affiliated with *tfdB* of Actinobacteria, Alpha- and Betaproteobacteria. *tfdB* diversity was similar or lower in the drilosphere than in bulk soil. Analyses of *tfdB* indicated the presence of novel and diverse 2,4-DCP degraders in the drilosphere and bulk soil. The collective data indicates that (a) earthworms stimulate the degradation of 2,4-DCP in soil by enhancing activity and growth of 2,4-DCP degraders, and (b) soils harbor diverse and novel putative 2,4-DCP-utilizing microorganisms.

METP013
Selective vertical mobilisation of plant-associated microbes in an agriculture soil after rainfall

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Extreme precipitation events promote the mobilisation of mobile organic particulate substances (MOPs), which can transport considerable amounts of carbon from plant-associated top soil layers to deeper mineral soils and also to groundwater. Substantial constituents of this efflux are assumed to be microbes. The composition of these translocated microbial communities and the fate of the transported biomass, however, are still unknown. At an experimental maize field in Göttingen, we collected soil and seepage water samples at different depths directly after a rainfall event in fall, as previously also done after snowmelt in winter. Using T-RFLP fingerprinting and pyrotag sequencing, we found that specific subsets of plant-associated *Bacteria* seem to be selectively mobilised from upper soil layers with seepage water. The bacterial communities in the mobile phase were clearly different from those in adjacent bulk soil samples across depths, whereas they were a lot more similar to the root surface communities above.
We are currently reproducing the analyses for the samples collected in September 2012. We speculate that the difference of fresh plant-derived labile carbon inputs in autumn will impose even more pronounced shifts in mobilised bacterial community structure. The composition of the translocated bacterial communities and their contribution to vertical carbon flux are assessed which extends our understanding of the organismic and trophic connectivity between soil compartments driven by groundwater recharge events.

METP014
Diversity, abundance and biogeography of H_2-oxidizing bacteria in tidal sediments

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Molecular hydrogen (H_2) is produced by various microbial processes and together with acetate it is the key intermediate in organic matter degradation in aquatic sediments. Many cultured bacteria oxidize H_2 under oxic or anoxic conditions. However, very little is known about identity and diversity of the mostly uncultured microorganisms that oxidize H_2 in nature. In this study we investigated the diversity, abundance and biogeography of uptake [NiFe] hydrogenases and of the corresponding microorganisms in tidal sediments along 2000 km of European coastline. Novel primers were designed targeting the gene for the large subunit of hydrogenases (*hupL*) of cultured and uncultured bacteria in order to generate clone libraries from genomic DNA. Analysis of *hupL*-transcripts and immunohistochemistry combined with fluorescence *in situ* hybridization (FISH) were applied to identify *hupL*-expressing bacteria. Using a polyclonal antiserum against an uptake [NiFe] hydrogenase targeted approximately 1.5% of the total cell counts in tidal sediments. Most of the antibody-labelled cells were identified by FISH as *Delta-* and *Gammaproteobacteria* accounting for up to 50% and 20%, respectively. In support of this the phylogenetic analysis of *hupL* sequences revealed a high diversity of mostly unknown, potentially H_2-oxidizing gamma- and deltaproteobacteria in tidal sediments. Regardless of the geographic region three major groups of potential H_2-oxidizers were identified. Two groups were related to sulfur-oxidizing, gammaproteobacterial endosymbionts of invertebrates and to sulfur-oxidizing *Thioalkalivibrio* sp. Among sulfate reducers most sequences affiliated with the δ1 endosymbiont of the *Olavius algarvensis* oligochaete. Using a single amplified genome of free-living relatives of the δ1 endosymbiont, we could link the *hupL* with the 16S rRNA gene sequence. Sediment incubation experiments confirmed a mainly sulfate reduction-based H_2 oxidation. We propose that endosymbiont-related sulfate reducers, which accounted for up to 10% of the total community, are the main H_2 oxidizers in tidal sediments. These organisms could therefore be essential for ecosystem functioning as they make fermentation processes possible by scavenging H_2.

METP015
Cellular quantification by CARD-FISH confirms that *Verrucomicrobia* are important members of the brackish bacterial community in the Baltic Sea.

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Changing salinity conditions represent a challenge to the physiology of aquatic bacteria. In a recent study on bacterial community compositions along the whole Baltic Sea salinity transect, applying 454 pyrosequencing, an adapted brackish community, including a dominant operational taxonomic unit (OTU) was identified. It is affiliated with "*Spartobacteria*", the second sub- group of the phylum *Verrucomicrobia*. This OTU seemed to be the most abundant organism particularly in the surface of brackish waters. Since there is no quantitative information about cellular abundance of *Verrucomicrobia* in the Baltic Sea, this study presents the first quantification along the whole salinity gradient.
We used the PCR independent method catalyzed reporter deposition-fluorescence in situ hybridization (CARD-FISH) to analyze samples, which were taken parallel to the samples used for the previously mentioned study. 70 samples from 34 stations were analyzed using probe VER47 for the whole phylum *Verrucomicrobia*, and the specific probe SPA714 for the subgroup "*Spartobacteria*" for a precise cellular quantification. In addition, a probe which exclusively targets the previously mentioned spartobacterial OTU was designed and applied.
In general, the relative abundance detected by CARD-FISH was about four times lower than the relative abundance detected by pyrosequencing. Cell numbers reached maxima of 2.4 x 10^5 cells x ml^{-1} detected with probe VER47 and 1.4 x 10^5 cells x ml^{-1} with probe SPA714, representing 12.3 % and 10.6 % of all EUB-positive cells, respectively. *Verrucomicrobia* were detected in nearly all samples, whereas almost no "*Spartobacteria*" could be detected in marine conditions and low abundances were detected in freshwater conditions. A Spearman rank correlation showed a significant positive correlation of "*Spartobacteria*" abundance with chlorophyll *a* concentration.

The general trend in the distribution of "*Spartobacteria*" as it was found by pyrosequencing could be confirmed in this study. Due to recent studies it is conceivable that Baltic Sea "*Spartobacteria*" are able to utilize polymer carbohydrates and thus might be associated with phytoplankton. This suggests that *"Spartobacteria"* find a niche in brackish areas of the Baltic Sea in which they overtake the function of polysaccharide degradation.

PHYV001
Insights into the physiology of *Geobacter metallireducens* at low growth rates

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Microbes in natural habitats are usually exposed to multiple substrates at extremely low concentrations, which lead to very low bacterial growth rates. Under carbon and energy limitation in chemostats, Enterobacteria utilize mixed substrates simultaneously. However, our knowledge on the physiology of environmentally relevant microorganisms at natural conditions is limited. The current study examines the physiology of the anaerobic, aromatics-degrading delta proteobacterium *Geobacter metallireducens* at very low growth rates and carbon limitation, supposedly close to natural. We expected expression of all available catabolic pathways at low growth rates. *G. metallireducens* was cultivated in batch and retentostat (continuous culturing with biomass retention) with different single and mixed carbon sources. The highest doubling time in retentostat was 328 hours (μ=0.0021 h^{-1}) in contrast to 4 hours in batch. In batch culture, *G. metallireducens* showed catabolite repression of utilization of aromatic compounds by acetate and ethanol, while in retentostat, benzoate and acetate were consumed simultaneously. Expression profiles of proteins in exponential growth phase in batch reflected the supplied substrates; however, some metabolic pathways were coexpressed, such as benzoate and butanoate metabolism on butyrate. At low growth rates with acetate or acetate plus benzoate limitation, there was a higher abundance of some proteins related to utilization of unavailable substrates, such as: alcohols, other aromatics, short chain fatty acids, and to unavailable electron acceptors, such as nitrate. Some signaling and chemotaxis related proteins were expressed at low growth rates only. In contrast to Enterobacteria, *G. metallireducens* does not express all available catabolic pathways upon extreme carbon limitation but only those which are involved in degradation of substrates that may be present in the respective environment (e.g., ethanol, butyrate, propionate, are released by anaerobic fermenting bacteria during the degradation of organic matter) together with a few aromatics-related proteins. Therefore, at low growth rates *G. metallireducens* establishes a specific survival strategy through a shift in physiology, directed towards the search for new environments and alternative substrates.

PHYV002
Anaerobic growth of aromatic compound-degrading bacteria with mixtures of succinate and benzoate: simultaneous or sequential utilization?

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In the presence of substrate mixtures, microorganisms display simultaneous or sequential substrate utilization, often depending on the concentration of the individual compounds. In batch cultures, the preferential utilization of the substrate supporting faster growth generally results in diauxic growth. Substrate preference is mediated by carbon catabolite repression (CCR), which is best understood in the selective utilization of carbohydrates by standard microorganisms such as *Escherichia coli* and *Bacillus subtilis*. So far, little is known about substrate preferences and CCR in non-standard, aromatic compound-degrading bacteria, especially under anoxic conditions.
This study investigated and compared the physiological response of various aromatic compound-degrading bacteria (succinate-adapted) to mixtures of succinate (5 mM) and benzoate (1 mM) under nitrate-reducing conditions. The tested bacteria include betaproteobacteria of the genus "*Aromatoleum*" (strains EbN1, HxN1, and ToN1) and *Thauera aromatica* K172, as well as the alphaproteobacterial *Magnetospirillum* sp. pMbN1. Distinct utilization patterns were observed that did not mirror the phylogenetic relationship, i.e. strains HxN1, ToN1 and K172 displayed monophasic growth with different co-utilization patterns, whereas strains EbN1 and pMbN1 displayed diauxic growth with preference of benzoate.
The unexpected preference of benzoate in succinate-adapted cells of strains EbN1 and pMbN1 was further investigated by two-dimensional difference gel electrophoresis (2D DIGE). In strain EbN1, the C_4-dicarboxylate periplasmic binding protein (DctP) of the C_4-dicarboxylate TRAP transporter (DctPQM) displayed the strongest decrease in abundance during benzoate utilization. In contrast, only marginal abundance changes were observed for DctP in strain pMbN1, agreeing with the consumption of 1 mM succinate already during benzoate utilization. Concomitantly, in strain pMbN1 the strongest decrease in protein abundance was observed for the E1 component of pyruvate dehydrogenase. This may suggest that in strain pMbN1 benzoate impedes the catabolism of succinate rather than its uptake, contrasting the repression of succinate uptake in strain EbN1.

PHYV003
Dinitrogen or ammonium? - A matter of speed.

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Two bacterial guilds compete for the electron acceptor nitrate: denitrifiers reduce nitrate to dinitrogen and nitrous oxide while dissimilatory nitrate reducers reduce nitrate to ammonium (DNRA). The outcome of this competition has an important environmental consequence: denitrification removes fixed nitrogen from the ecosystem, while DNRA keeps fixed nitrogen available for primary production. The environmental conditions that select for the one or the other are presently debated and actively researched. In this study heterotrophic nitrate respiring communities from sediments of an intertidal flat active in nitrogen cycling were enriched in chemostats under different constant conditions. The conversion of nutrients was followed by mass balancing of nitrogen and carbon compounds. The microbial community composition was analyzed by Illumina and Ion torrent sequencing. With nitrite as electron acceptor (the branching point of the two pathways with respect to nitrogen species and the enzymes involved) denitrification became prevalent under all conditions tested (e.g. various electron donors and different ratios of electron donor to acceptor as well as growth rates). In contrast, under the supply of nitrate the growth rate discriminated between the two pathways leading to denitrification at a higher growth rate and to DNRA at a lower growth rate. Overall, we showed that not thermodynamics (e.g. the ratio of electron donors to acceptor) but kinetics discriminate between the two nitrate reduction pathways.

PHYV004
Interactions of sulfur and nitrogen cycle bacteria in a marine, laboratory scale model system

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Anaerobic ammonium oxidizing (anammox) bacteria of the genus '*Candidatus* Scalindua sp.' are so far the only anammox bacteria detected in oxygen limited marine ecosystems. Like other anammox bacteria they derive their energy for growth from the conversion of NH_4^+ and NO_2^- into dinitrogen gas, thereby constituting a significant sink for fixed nitrogen in the ocean. The most important oxidant and reductant at the chemocline of marine anoxic basins are oxygen and hydrogen sulfide, respectively.. Sulfide is toxic and has been shown to have a strongly negative effect on anammox activity. It can be found in marine sediments, within the deeper layers of the oxygen minimum zone or in anoxic marine basins. Its widespread occurrence raises the question whether anammox can tolerate short- and long-term exposure to sulfide (reversible inhibition) and/or might coexist with bacteria capable of sulfide oxidation (such as autotrophic denitrifiers), broadening the ecological niche for anammox by detoxification. Additionally, denitrification coupled to anaerobic sulfur oxidation converts NO_3^- to NO_2^- in an initial step, followed by its reduction to NO, N_2O and ultimately the production of N_2. Leaking intermediates and/or end products

of partial denitrification could be substrates for anammox bacteria. Here we investigate the possibility of such an interaction in a laboratory scale model system under controlled conditions. Gammaproteobacterial sulfide oxidizers were enriched under anaerobic conditions and inoculated with anammox biomass (30%) when the culture was stable. Sulfide, ammonium and nitrate were supplied as the only substrates. A stable co-culture was obtained after several weeks. Cells were counted by FISH and qPCR, and substrates determined colorimetrically and by HPLC. Activity of the culture was determined with GCMS by following the production of $^{29}N_2$ and $^{30}N_2$ from labeled substrates. DNA of the co-culture was extracted and the metagenome was sequenced by Ion Torrent technology. The experiment showed that a stable co-culture of nitrate-reducing sulfide oxidizers and anammox bacteria is possible, and could play an important role in OMZs.

PHYV005

The role of archaea in nitrate-dependent anaerobic oxidation of methane

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The first described enrichment culture capable of anaerobic oxidation of methane (AOM) coupled to nitrite/nitrate reduction was a consortium of bacteria, *Methylomirabilis oxyfera* (~80%), and archaea (~10%)[1], which was termed AOM-associated archaea (AAA)[2]. AAA were assumed to function analogously to the anaerobic methanotrophic archaea (ANME) in sulfate-reducing consortia. The AAA would perform reverse methanogenesis, and shuttle intermediates to *M. oxyfera* that reduce nitrate/nitrite to N_2. However, after prolonged incubation with elevated nitrite the AAA disappeared from the enrichment culture, and *M. oxyfera* was shown to be able to oxidize methane without an archaeal partner[3]. Nevertheless, we were able to maintain enrichment cultures with about equal numbers of AAA and *M. oxyfera* on nitrate and methane. The mixed culture showed nitrate-dependent methane oxidizing activity, and N_2 and CO_2 were detected as the main products, although transient nitrite accumulation was often observed. *M. oxyfera* bacteria alone were unable to use nitrate for methane oxidation and did not convert nitrate to nitrite, indicating that AAA may be responsible for this conversion. DNA was extracted from the AAA/*M.oxyfera* mixed culture and sequenced by two ion torrent runs, yielding 1.6 Gb metagenomic information. De novo assembly resulted in 400 contigs with high coverage. Next to the entire methanogenesis pathway, several genes putatively involved in nitrate and nitrite reduction could be retrieved. Physiological and biochemical experiments are now being performed to elucidate the mechanism of nitrate dependent methane oxidation. Because nitrate is an important electron acceptor in many freshwater environments, archaeal denitrifying AOM is of great interest for the understanding of the biogeochemical cycles of methane and nitrogen.

[1]Raghoebarsing et al. (2006) *Nature* 440: 918-921
[2]Knittel & Boetius (2009*) Ann Rev Microb* 63: 311-334
[3]Ettwig, KF et al. (2008) *Env Microb* **10**(11): 3164-3173

PHYV006

Comparative oxidation kinetics of *Nitrospira* and *Nitrobacter* pure cultures

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Aerobic nitrite oxidation is the second step of nitrification, a process catalyzed by nitrite-oxidizing bacteria (NOB). NOB consist of six genera, of which *Nitrobacter*, *Nitrospira* and the recently discovered *Nitrotoga* and *Nitrolancetus* were detected in activated sludge of wastewater treatment plants. Since these slow growing, chemolithoautotrophic bacteria use nitrite as their sole energy source, growth is directly linked to the availability of this substrate and the kinetics of its oxidation. Interestingly, different genera were found in the same municipal wastewater treatment plant, which leads to the hypothesis of a small scale heterogeneity in this habitat. Additionally, also members of two different sublineages of the predominant *Nitrospira* were present in the same WWTP, suggesting niche differentiation even within the same genus.
To further investigate the substrate-dependent niche partitioning among nitrite-oxidizers, the aim of this study was an advanced comparative analysis of NOB oxidation kinetics and growth characteristics. The measurements of nitrite-dependent oxygen consumption were carried out directly in pure cultures by a precise microsensor-based respiration system.
The measurements revealed a discrepancy of oxidation kinetics between *Nitrospira* and *Nitrobacter* spp., but also within these genera between species and sublineages. Members of the genus *Nitrobacter* (r-strategists) showed high nitrite turnover rates (V_{max}) of 61 (*Nb. hamburgensis*; soil), 81 (*Nb. winogradskyi*; soil) and 179 μmol N/mg protein/h (*Nb. vulgaris*; activated sludge). In contrast, members of the genus *Nitrospira* (K-strategists) presented lower maximum nitrite turnover rates under the applied conditions. Here, values of 48 (*Nitrospira defluvii*; activated sludge) and 22 μmol N/mg protein/h (*Ns. moscoviensis*; heating water) were obtained. Furthermore, the results confirmed significant differences in the substrate affinities (K_m) with a sixfold higher affinity for nitrite of *Ns. defluvii* (K_m 11 μM) compared to *Nb. vulgaris* (K_m 66 μM). It can be speculated that the K_m values reflect the specific availability of nitrite in the corresponding microenvironment. These results give an extended view on oxidation kinetics of nitrite-oxidizing bacteria and substrate affinities are presented as an important factor providing niche differentiation.

PHYV007

NO-induced dispersion of Pseudomonas aeruginosa biofilms is mediated by the phosphodiesterase NbdA

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Pseudomonas aeruginosa is a ubiquitous bacterium but commonly known as nosocomial pathogenic organism. This is especially due to its ability to form biofilms. The last step of the biofilm cycle is the dispersion of planktonic cells to colonise new habitats. Multiple environmental cues like heavy metals, nutrient availability or nitric oxide (NO) are known to trigger biofilm dispersion through stimulating of c-di-GMP degrading phosphodiesterase (PDE) activity. PDEs are distinguished by their EAL domain whereas GGDEF domains harbour the opposite c-di-GMP forming diguanylate cyclase (DGC) activity. While high concentrations of c-di-GMP are associated with a sessile lifestyle and the formation of biofilms, low amounts are associated with high motility.
Here we describe the multidomain protein PA3311 that consists of an GGDEF-EAL- fusion and an N-terminally located membrane anchored MHYT sensor domain. The latter is proposed to sense diatomic gases. Different biochemical analyses employing truncated protein variants lacking the sensor domain revealed a PDE activity of PA3311. The GGDEF motif is degenerated and shows no DGC activity but is necessary for full PDE activity through binding GTP. Phenotypical analysis of a knock-out mutant confirmed the PDE activity by reduced swarming behaviour based on decreased rhamnolipd production. Furthermore, the mutant displayed an increased biofilm attachment and most importantly the inability to respond to NO-induced biofilm dispersion. This is the first characterised PDE that is directly involved in NO regulated biofilm dispersion. Hence we named PA3311 NdbA for NO-induced biofilm dispersion locus A.

PHYV008

Bleach turns a conserved *Escherichia coli* protein from a family with a diverse set of functions into a highly efficient chaperone

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The *Escherichia coli* Protein RidA is a member of the highly conserved YER057c/yjgf/Uk114 family of proteins. Members of this family have a multitude of assigned functions, among others these include translational inhibition, activation of proteinases, and nuclease function (Schmiedeknecht *et al*. 1996, Melloni *et al*. 1998, Morishita *et al*. 1999). RidA in *Salmonella* has been recently shown to function as an enamine/imine deaminase in the branched chain amino acid pathway (Lambrecht *et al*., 2012).
Here we show that, while it has an enamine/imine deaminase activity in *E. coli* as well, RidA gets efficiently converted to a chaperone upon reaction with sodium hypochlorite (household bleach) or chloramines. The activated RidA chaperone has a wide range of substrates and presumably helps stabilizing proteins that precipitate in the presence of chlorine species *in vivo*. Although thiols are a known target of chlorine, the activation we observe is independent of the amino acid cysteine: a cysteine to serine mutant of RidA is still activated by chlorine species. Nevertheless, the chlorine-based modification is reversible by reduction with ascorbic acid and DTT *in vitro*. A reconstituted thioredoxin/thioredoxin reductase/NADPH system in catalytic quantities can inactivate the RidA

chaperone as well. To our knowledge, we describe here for the first time a chlorine-based mechanism of protein activation that is independent of cysteine and a new regulatory de-chlorination function for the thioredoxin system.

PHYV009

Microbial degradation of rubber by *Gordonia polyisoprenivorans*

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The increasing production volume of poly(*cis*-1,4-isoprene) rubber (over 26 million tons in 2011) leads to huge challenges in waste management. The polymer is mainly produced synthetically (synthetic rubber) or harvested from the rubber tree *Hevea brasiliensis* (natural rubber). The actinomycete *Gordonia polyisoprenivorans* strain VH2, isolated from soil of a rubber tree plantation, was found to be one of the most effective natural and synthetic rubber-degrading bacteria (1). Hitherto, only little is known about the bacterial degradation of rubber, a capability which seems to be limited mainly to actinomycetes (2). In order to shed light on the mechanisms of degradation the genome of strain VH2 was sequenced and annotated (3). A comparison to the genomes of other rubber degrading and non-rubber degrading actinomycetes, generation of transposon-induced as well as of deletion mutants enables us to predict a pathway for the microbial degradation of rubber.

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PHYV010

The catalytic haem centre of extracellular rubber oxygenase RoxA is oxygenated

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RoxA is a *c*-type dihaem cytochrome secreted by *Xanthomonas* sp. 35Y during growth on natural rubber. RoxA cleaves poly(*cis*-1,4-isoprene) oxidatively to 12-oxo-4,8-dimethyltrideca-4,8-diene-1-al (ODTD) by a dioxygenase mechanism. Recombinant RoxA was expressed in *Xanthomonas* sp. and purified from the culture supernatant. Chemical reduction and anoxic reoxidation of purified RoxA caused changes in the optical and EPR-spectrum that were indicative for an originally dioxygen-ligated haem in RoxA. This assumption was supported by determination of UV-Vis signals of RoxA that indicated removal of dioxygen by exposure to anoxic conditions or by addition of exogenous haem ligands such as imidazole which occupy a formerly oxygenated 6th coordination site [1]. The observed spectral changes were restricted to the N-terminal haem group which can be present in high- and low-spin states and was identified to be the catalytic centre [1, 2]. Further evidence for an oxygenated state was obtained by analysis of the 3-dimensional structure of RoxA at 1.8 Å that showed the presence of a di-atomic molecule as distal heme ligand. The assumed oxy-haem revealed a remarkable stability that is in contrast to other known haem oxygenases. We explain the stability by the hydrophobic character of the active site heme cavity that is built up by A251, I252, F301, A316, F317 and by the absence of any acid-base catalysts. Furthermore, comparison of the spectroscopic properties of RoxA variants with single haem cavity mutations suggest an important role of a catalytically essential phenylalanine residue (F317) [2] in stabilisation of the oxygenated state.

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PHYV011

Degradation of the acyl side chain of the steroid compound cholate in *Pseudomonas* sp. strain Chol1 proceeds via an aldehyde intermediate

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Steroids are ubiquitous natural compounds with diverse functions in eukaryotes. In bacteria, steroids occur only as rare exceptions but the ability of transforming and degrading steroids is widespread among bacteria.
We investigate bacterial steroid degradation using the bile salt cholate as model compound and *Pseudomonas* sp. strain Chol1 as model organism. Cholate degradation is initiated by oxidative reactions at the A-ring followed by cleavage of the side chain attached to C17. Two mutant strains of strain Chol1 with defects in the genes *skt* and *acad* are blocked in degradation of the acyl side chain and accumulate the C24-steroid (22E)-7a,12a-dihydroxy-3-oxochola-1,4,22-triene-24-oate (DHOCTO) and the C22-steroid 7α,12α-dihydroxy-3-oxopregna-1,4-diene-20-carboxylate (DHOPDC) as dead end products, respectively. The structures of those intermediates indicate that side chain degradation occurs via a pathway similar to b-oxidation of fatty acids.
We investigated the degradation of the acyl side chain *in vitro* using cell extracts of strain Chol1 in the presence of co-factors (CoA, ATP and NAD^+) and DHOCTO and DHOPDC as substrates. DHOCTO was completely transformed to 7α,12α-dihydroxy-androsta-1,4-diene-3,17-dione (12α-DHADD) and 7α-hydroxy-androsta-1,4-diene-3,12,17-trione (HADT) as end products indicating complete removal of the acyl side chain *in vitro*. The same products were formed with DHOPDC as substrate. The 12-keto compound HADT was further transformed into 12β-DHADD in an NADPH-dependent reaction.
When NAD^+ was omitted from assays with DHOCTO a new product was formed and identified as 7α,12α-dihydroxy-3-oxopregna-1,4-diene-20S-carbaldehyde (DHOPDCA). This aldehyde was further oxidized to DHOPDC in the presence of NAD^+ and to DHOPDC-CoA in the presence of NAD^+, CoA and ATP. DHOPDCA was also detected in culture supernatants of strain Chol1 growing with cholate [1].
These results indicate that degradation of the C5-acyl side chain of cholate does not proceed via a classical β-oxidation but via a free aldehyde that is oxidized to the corresponding acid. The reaction leading to the aldehyde is presumably catalyzed by an aldolase encoded by the gene *skt* that was previously predicted as a β-ketothiolase.
Based on the genome sequence of strain Chol1 we are currently inactivating further genes with probable functions in side chain degradation [2].

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PHYV012

Novel phospholipid biosynthesis pathways identified in *Xanthomonas campestris*

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Bacterial membranes are typically composed of phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and cardiolipin (CL). However, about 10% of all bacteria are assumed to produce the typical eukaryotic membrane lipid phosphatidylcholine (PC) which plays an important role in symbiotic and pathogenic interactions [1, 2, 3]. Two PC biosynthesis pathways are known in bacteria. The three step methylation of PE via the intermediates monomethyl-PE (MMPE) and dimethyl-PE (DMPE), which is catalysed by phospholipid *N*-methyltransferases (Pmt) using *S*-adenosylmethionine (SAM) as methyldonor. In a second pathway a PC synthase (Pcs) uses choline and CDP-diacylglycerol (CDP-DAG) as substrates for PC formation [2].
Here, we investigated the phospholipid biosynthesis in the black rot causative agent *Xanthomonas campestris*. A bioinformatic approach revealed one annotated *pmt* gene (*pmtA*) suggesting a methylation pathway for PC biosynthesis in this organism. Thin layer chromatography analysis of the membrane lipids demonstrated that *Xanthomonas* is able to produce significant amounts of MMPE and small amounts of PC. Heterologous expression of the *Xanthomonas pmtA* gene in *E. coli*, which is not able to produce methylated PE derivatives, resulted in MMPE, but not in PC synthesis. A *Xanthomonas* Δ*pmtA* strain was deficient in MMPE formation,

but still produced small amounts of PC in rich medium, implying a Pcs-like pathway. Feeding experiments with different PC precursors like choline and glycerophosphocholine (GPC) demonstrated a GPC-dependent PC synthesis in *Xanthomonas* indicating a novel PC biosynthesis pathway in bacteria. Via [^{14}C]-ethanolamine (EA) feeding experiments and subsequent analysis of the membrane lipid composition we observed a EA-dependent PE synthesis in *Xanthomonas*. The corresponding gene *xc_0186*, encoding a putative CL synthase, was identified via mutagenesis. Expression analyses of *xc_0186* in *E. coli* revealed an enzyme capable of CL and PE synthesis suggesting a dual role of Xc_0186 for phospholipid biosynthesis in *Xanthomonas*. The physiological role and biochemical verification of Xc_0186 functionality and substrate specificity will be analysed in future studies.

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PHYV013

Analysis of small acid-soluble spore proteins in *Clostridium acetobutylicum*

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Small acid-soluble spore proteins (SASP) are non-specific, double-stranded DNA-binding proteins in dormant spores of the genera *Bacillus* and *Clostridium*. They are expressed in the developing forespore during stage III of sporulation. These highly conserved proteins are able to saturate the spore DNA and thus protecting it against environmental insults like UV radiation, heat or peroxides. During the process of germination SASPs are degraded by germination proteases (GPR) and serve as amino acid supply for the protein synthesis during the spore outgrowth (1).
In the genome of the apathogenic *Clostridium acetobutylicum* five putative SASPs are predicted (2). Insertional mutants of these five SASPs were generated and analysed with respect to their sporulation rate, germination rate and spore resistance. The sporulation of the generated mutants were not influenced, but the abilities of spore germination were altered in four of five SASP mutants compared to the parental strain. Most interestingly the deletion of one SASP leads to a completely loss of germination capabilities of spores produced by this strain. On the other hand three SASPs mutants showed a decreased resistance to heat stress.
We started to investigate the DNA protection mechianism of the individual proteins. Therefore heterologous overexpression strains of *E. coli* were generated and the SASPs were enriched. DNA-binding assays revealed that these proteins are able to bind and to protect DNA.

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PHYV014

Oxygen and Nitrate Respiration in Spores of *Streptomyces coelicolor*

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Streptomycetes are high-GC Gram-positive actinobacteria that undergo a complex life cycle with stages including vegetative hyphae, hydrophobic aerial hyphae and spore formation. Metabolism in spores must be maintained at a low level to retain viability over long periods. Long-term survival of spores requires that essential metabolic pathways to cope with anaerobic conditions are also available. Recent studies revealed that despite being an obligate aerobe, *Streptomyces* is able to reduce nitrate to nitrite during periods of hypoxia. Nitrate reduction is catalyzed by the respiratory nitrate reductases (Nar), which are energy-conserving, multi-subunit, membrane-associated enzymes. *S. coelicolor* synthesizes three Nar enzymes. These Nars are not redundant but rather appear to have distinct functions in the developmental program of the bacterium [(1)]. Using various biochemical and physiological methods we could demonstrate that resting spores harbour an active Nar. Freshly harvested spores reduced nitrate to nitrite at significant rates without addition of an exogenous electron donor. Nitrate reduction only occurred in the absence of oxygen. Nar enzyme activity was detected in crude extracts of spores and by direct staining after native PAGE. Analysis of defined knockout mutants demonstrated that this activity was exclusively due to Nar1. Via western blot analysis we could show that Nar1 is always present in dormant spores. We could also demonstrate that crude extracts of spores have an oxidase activity. Taken together, these data indicate that dormant spores retain the ability to respire using either oxygen or nitrate as electron acceptor.

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PHYV015

The *Escherichia coli* SLC26 homologue YchM (DauA) is a C_4-dicarboxylic acid transporter

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The ubiquitous SLC26A/SulP (Solute carrier 26/ Sulphate permease) family is a gene family of highly versatile anion transporters/channels with intriguing roles in normal cell physiology and human pathophysiology. These versatile exchangers have an expanding substrate repertoire of monovalent and divalent anions, ranging from halides to carboxylic acids. However, the low level of sequence similarity among the family members does not allow reliable prediction of function or substrate specificity. The functional importance of this family is illustrated by the fact that several inherited human diseases are caused by mutations in SLC26 genes, mutations in the sulP genes lead to starvation syndrome in plants and auxotrophic phenotypes in fungi. While the amount of information on eukaryotic members of the SCL26A/SulP family constantly increases, the role of the prokaryotic homologues remains unclear. Many bacterial species have been shown to express multiple SulP homologues. However, none of the bacterial SulP proteins have been shown to play an active role in sulphate transport.
We used a combination of genetic, microbiological and biochemical techniques to investigate the physiological role of YchM, the *E.coli* SLC26 homologue.
We have proved that YchM, is a C_4- dicarboxilic acid transporter, (succinate, funarate, aspartate). Therefore we have re-named ychM as dauA (for dicarboxylic acid uptake system A). Additionally, DauA appears to form a multiprotein complex at the membrane with DctA, a well characterised aerobic C_4-dicarboxilic acid transporter, and with DcuS, a membrane-bound sensor protein that senses dicarboxilic acid availability in the external medium and accordingly regulates the expression of genes involved in dicarboxilic acid metabolism.
This is the first report that a SLC26/SulP protein acts as a C_4-dicarboxylic acid transporter, an unexpected new function for a prokaryotic member of this transporter family.

PHYV016

Drastic physiological and morphological variation in strain *Pseudovibrio* FO-BEG1 induced by phosphorus limitation

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Pseudovibrio sp. FO-BEG1, an alphaproteobacterium closely related to *Pseudovibrio denitrificans* is metabolically versatile and shows a potential to produce secondary metabolites (Bondarev, 2012). In the present work we investigated the physiological response of strain FO-BEG1 to phosphorus (P) limitation. P is a crucial component in all living organism, as many of the components and the regulation processes in the cell require this element. It has been shown that P limitation not only affects the bacterial P metabolism, leading to up-regulation of P-acquiring systems, but triggers a severe variation of the overall bacterial physiology, including the production of secondary metabolites. In order to understand the effect of the limitation of phosphorus on the physiology and the secondary metabolite production of *Pseudovibrio* FO-BEG1, we compared the physiology of this strain under P limited and P surplus conditions, using classical microbiological and proteomic approaches. The physiological experiments, supported by the proteomic data, demonstrated that under phosphorus-limited conditions strain FO-BEG1 induced the synthesis of high-affinity transporter systems and enzymes for uptake and degradation of organic phosphorus-containing molecules. Furthermore, we could show that the cells of strain FO-BEG1 accumulate internal inclusions of carbon and phosphorus in the form of polyhydroxyalkanoates and polyphosphate, respectively. Intriguingly, we

also observed an increased uptake of iron and cobalt, indicating a higher need of these nutrients for so far unknown cellular processes. This condition also triggers cell elongation and rearrangement of the polar lipids of the cells, substituting phosphorus containing lipids with phosphorus-free lipids. Moreover P limitation triggers the production of the potent antibiotic tropodithietic acid (TDA) together with a so far not characterized yellow-orange compound. In conclusion, the P limitation response of strain FO-BEG1 is characterized by a drastic change in the cellular metabolism, including up-regulation of proteins for phosphorus acquisition and the production of bioactive compounds to successfully compete for the scarce nutrient.
Bondarev V, 2012, PhD thesis

PHYV017

Guided *de novo* corrinoid biosynthesis of Benzimidazolyl-Norcobamides in the organohalide-respiring *Sulfurospirillum multivorans*

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Reductive dehalogenation of organohalides is detected in a phylogenetically diverse group of anaerobic bacteria and relies on the activity of reductive dehalogenase enzymes (Rdhs). A corrinoid-cofactor (Vitamin B_{12} derivative) is located at the active site of almost all Rdhs purified and biochemically analyzed so far. The heterogeneous group of reductively dehalogenating bacteria can be divided into organisms able to synthesize corrinoids *de novo* (e. g. *Desulfitobacterium* spp., Firmicutes) and others depending strictly on corrinoid salvaging from the environment (e. g. *Dehalococcoides* spp., Chloroflexi). Up to date little is known about the structural diversity of corrinoids present in Rdhs from different anaerobic bacteria.
The epsilonproteobacterium *Sulfurospirillum multivorans* is able to conserve energy via electron-transport phosphorylation coupled to the corrinoid-dependent reductive dehalogenation of tetrachloroethene (organohalide respiration). The structural analysis of the corrinoid cofactor of the tetrachloroethene (PCE) reductive dehalogenase (PceA) revealed a unique Norpseudo-B_{12} (Adeninyl-Norcobamide) produced *de novo* by the organism [1]. Besides the common structural characteristics of Vitamin B_{12} derivatives, e. g. the contracted tetrapyrrole (corrin ring) with the cobalt ion in the center, the corrinoid cofactor of PceA harbors a modified nucleotide loop with adenine as lower ligand base of the cobalt ion. In addition to adenine, most frequently 5,6-dimethylbenzimidazole (DMB) is found as lower ligand base in corrinoids extracted from natural sources.
In this study we investigated the impact of benzimidazole analogues added to the growth medium of *S. multivorans* on the organohalide respiration. All benzimidazole analogues tested (10 μM each) had a negative effect on PceA activity, while DMB caused the highest decrease in growth of *S. multivorans* with PCE. Using LC-MS and H^1-NMR the formation of Nor-B_{12} (5,6-Dimethylbenzimidazolyl-Norcobamide) in DMB-amended cultures was proven. Both, Norpseudo-B_{12} and Nor-B_{12} have been extracted from purified PceA, implicating a negative effect of the DMB-moiety on the enzyme activity rather than on cofactor binding. This assumption was supported by the reduced abiotic dechlorination rate of Nor-B_{12} compared to Norpseudo-B_{12}.

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PHYV018

Respiratory complex iron-sulfur molybdoenzymes (CISM): diversities and similarities of CISM with membrane-bound subunits of the PsrC/NrfD-type

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CISM are a widespread class of enzymes that have essential functions in energy conserving metabolisms of prokaryotic organisms [1]. Their physiological functions are fundamental and include catalytic key reactions in carbon, nitrogen, arsenic, selenium and sulfur metabolism. CISM are soluble or membrane-bound enzymes that are composed of one or multiple subunit(s). Apart of the well-known dissimilatory formate dehydrogenase and nitrate reductase from *Escherichia coli* little information on members of the membrane-bound type of CISM is available. Based on their modular composition and the sequence homology/similarity of the corresponding subunits an unambiguous assignment of specific enzyme functions is not possible. This dilemma is reflected by the quantity of erroneously annotated CISM in genome databases. From the bioenergetic point of view the membrane-bound subunits of CISM are of special interest. Those proteins containing no haem group belong to the PsrC/NrfD-protein family. The major function of these subunits is coupling of the enzyme reactions to the redox-reaction of menaquinone/menaquinol. However, almost nothing is known about the corresponding proton-uptake or -release pathways although this is an essential prerequisite for the elucidation of the bioenergetics of the respiratory processes. PsrC/NrfD-proteins show significant differences based on bioinformatics analysis of their primary structures, their number of predicted trans-membrane helices and their calculated protein structures. The key enzyme to answer open questions is the polysulfid-reductase (Psr) of *Wolinella succinogenes* [2, 3]. So far, functional results obtained with this enzyme (specificity for 8-methyl-menaquinone in electron-transfer reaction and putative proton-bifurcation reaction during reoxidation of 8-methy-menaquinol) are in contrast to hypothesis inferred from the single known high resolution structure model of a CISM with a PsrC/NrfD-type protein whose enzymatic function is unclear [4]. Diversities and similarities of CISM with such kinds of membrane-subunits will be discussed.

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PHYV019

Specific Mo-cofactor containing tertiary C-25 hydroxylases involved in anoxic degradation of steroids with variations in the side chain.

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Cholesterol and related plant sterols like sitosterol are the most abundant steroid compounds in the environment. These ubiquitous steroids are recalcitrant to degradation owing to their low solubility in water, complex ring structure, the presence of quaternary carbon atoms, and the low number of functional groups. Nevertheless, they serve as growth substrates for bacteria. In aerobic steroid catabolism, oxygenases hydroxylate steroids at different positions and cleave the steran ring system. In contrast, in anaerobic bacteria, hydroxylating enzyme reactions use water instead of oxygen as cosubstrate[1]. During anaerobic cholesterol catabolism in *Sterolibacterium denitrificans* hydroxylation of the C-25 tertiary atom is catalyzed by a specific Mo-cofactor containing enzyme belonging to the DMSO dehydrogenase family[2].
The analysis of the draft genome of the *S. denitrificans* revealed seven genes coding for related Mo-containing hydroxylases putatively involved in anoxic steroid metabolism. A second Mo-cofactor containing enzyme is specifically involved in C-25 hydroxylation during sitosterol degradation. The molecular and kinetic properties of this previously unknown Mo-containing hydroxylase involved in anoxic sitosterol degradation have been characterized. Evidence is obtained that *S. denitrificans* contains at least three different C-25 side chain hydroxylases involved in degradation of steroids with alternative side chain modifications.

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PHYV020

Mechanistic and stereochemical investigations on the transformation pathways of hydrocarbons in *n*-alkane-degrading, anaerobic bacteria

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In order to obtain a better mechanistic understanding of alkane degradation in anaerobic bacteria, we investigated in more detail the metabolic pathway of *n*-hexane oxidation in the denitrifying bacterium strain HxN1.[1,2] Here, we present an overview of recent analyses contributing to our understanding of *in vivo* mechanisms in anaerobic alkane activation, with particular emphasis of stereochemistry. Using stereospecifically deuterated *n*-hexanes we showed that the alkane-activating glycyl radical enzyme stereospecifically removes the pro-*S* H-atom from C-2 of the *n*-alkane.[3] This process may be formulated as proceeding either via a discrete hex-2-yl radical or synchronously with inversion of configuration at C-2 of hexane. We proposed that the alkylsuccinate is epimerized at C-2 in preparation for the subsequent intramolecular rearrangement, most likely at the CoA-thioester level.
Additionally, we investigated the substrate range of the alkylsuccinate-forming enzyme and the stereochemistry of the formed co-metabolites. In cultures of strain HxN1 growing with defined mixtures of *n*-hexane and toluene, the transformation of toluene to benzoate via the *n*-alkane-pathway was demonstrated.[4] This is in contrast to the toluene metabolism via modified β-oxidation in alkylaromate-degraders, which are unable to activate *n*-alkanes. In an analogous experiment with butyl- and pentylbenzene, strain HxN1 regiospecifically activated the aromatic hydrocarbon at the ω-2 position of the side chain, although the benzyl position offers an energetically less challenging target. These results provide evidence for the pronounced evolutionary adaptation to specific substrates among such anaerobes. During incubation of *n*-alkane-degrading bacteria with crude oil, assemblages of metabolites deriving from aliphatic and aromatic hydrocarbons were formed. The stereochemistry of these metabolites, also for 2-benzylsuccinate derivatives, is in accordance with that found for *n*-hexane degradation, but differs from that observed in aromatic hydrocarbon-degrading anaerobic bacteria.

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PHYV021

Structure/Function Relationship in Sulfur Oxygenase Reductases from Thermophilc Archaea and Mesophilic Bactreria

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Soluble sulfur oxygenase reductases (SOR) are the initial enzymes in sulfur oxidation of the thermoacidophilic archaeon *Acidianus ambivalens* (AaSOR) and of the mesophilic Gammaproteobacterium *Halothiobacillus neapolitanus* (HnSOR). Database searches showed that the 16 SOR proteins from predominantly chemolithoautotrophic bacterial and archaeal species form a coherent protein family with high mutual similarities.
The HnSOR produced in *E. coli* had an optimal enzyme activity at pH 8.4 (range 5-11) and 80°C (range 10-99°C) and a 4-10fold higher specific activity compared to the AaSOR (42 U/mg protein). Sulfite, thiosulfate and hydrogen sulfide were formed at varying stoichiometries in a range between pH 5.4 and 11 (optimum pH 8.4). CD spectroscopy and dynamic light scattering showed that the HnSOR adopts similar secondary and quaternary structures as the AaSOR. When crystallized, the HnSOR diffracted to 2.9 Å resolution. Preliminary structure refinement showed that the HnSOR is a globular, hollow, 24-subunit protein with 432-point group symmetry like the AaSOR. The active sites in each subunit of both enzymes comprise a mononuclear low-potential non-heme iron and a cysteine persulfide in a spacious pocket. Structural differences appeared in the second coordination sphere and in the presence of a "back exit" in the active site pockets of the HnSOR. Pores at the non-crystallographic 4-fold and 3-fold symmetry axes of both enzymes provide pathways for substrate access and product exit. Pore opening lead to an increase in activity. Binding of Zn^{2+} ions as an inhibitor seem to block the putative pathway of product exit at the 3-fold symmetry axis.
A hydrogen-bonding network in the second coordination sphere surrounding the metal ion presumably contributes to the low reduction potential. Mutagenesis of surrounding residues in the AaSOR reduced but did not abolish SOR activity. Derivatives with the Fe^{3+} ion exchanged for Co^{2+}, Mn^{2+}, Ni^{2+}, and Ga^{3+} resulted in active enzyme suggesting that a valence change does not occur during oxygen activation. We propose a reaction mechanism involving a hydroxyl-catalyzed nucleophilic cleavage of cysteine-bound polysulfide as the initial step. The resulting polysulfenic acid is the substrate and reductant for O_2 and for formation of polysulfonic acid as the primary product.

PHYV022

An electron bifurcating caffeyl-CoA reductase in the acetogenic bacterium *Acetobacterium woodii*

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The acetogenic bacterium *Acetobacterium woodii* conserves energy by reduction of the electron acceptor caffeate with electrons derived from molecular hydrogen according to:
H_2 + ADP + P_i + caffeate --> hydrocaffeate + ATP
ATP is synthesized by a chemiosmotic mechanism with Na^+ as coupling ion and Na^+ translocation is driven by the exergonic electron flow from reduced ferredoxin to NAD^+, catalyzed by the ferredoxin:NAD^+ oxidoreductase (Rnf) (1). The ferredoxin has a very low redox potential ($E_0' \approx$ -500 mV) and, therefore, its reduction with physiological electron donors such as H_2 or NADH is highly endergonic. Thus, ferredoxin reduction must be coupled to an exergonic reaction. One candidate is the already established electron bifurcating hydrogenase (2). Another candidate reaction is the NADH-dependent caffeyl-CoA reduction which is exergonic. We have purified the caffeyl-CoA reductase from *A. woodii* that forms a complex with an electron transfer flavoprotein. The enzyme complex is predicted to have apart from FAD two [4Fe4S]-cluster as cofactor which is consistent with the experimental determination of 4 mol FAD, 9 mol of Fe and 9 mol of acid labile sulfur. With NADH as donor the complex catalyzed caffeyl-CoA reduction with low activities, but this reaction was highly stimulated by addition of ferredoxin. Spectroscopic analyses revealed that ferredoxin and caffeyl-CoA were reduced simultaneously in a stoichiometry of 1.3 : 1. Apparently, the caffeyl-CoA reductase/Etf complex of *A. woodii* uses the novel mechanism of flavin-dependent electron bifurcation (3) to drive the endergonic ferredoxin reduction with NADH as reductant by coupling it to the exergonic NADH-dependent reduction of caffeyl-CoA. The energetics of caffeate respiration is discussed.

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PHYV023

Structure and function of the unique membrane-embedded rotor of the Na^+ F_1F_O ATP synthase of *Acetobacterium woodii*

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The Na^+ F_1F_O ATP synthase of the anaerobic, acetogenic bacterium *Acetobacterium woodii* is unique because it has a membrane-embedded hybrid rotor made by an 8 kDa bacterial F_O-like *c* subunit with two transmembrane helices, and an 18 kDa eukaryal V_O-like *c* subunit with four transmembrane helices but only one binding site. We purified the *c* ring, crystallized it and determined its structure at 2.1 Å resolution. As evident from the 3D structure and from earlier biochemical experiments, both types

of *c* subunits are incorporated into the *c* ring in a 9 : 1 (F_O:V_O) stoichiometry (1). To begin a molecular study, we cloned the entire *atp* operon into the expression vector pTrc99A (Amersham Bioscience), introduced a His_6-tag at the N-terminus of the β subunit and transformed the resulting plasmid into the ATP synthase negative *Escherichia coli* strain DK8 (2). The heterologeously produced ATP synthase was purified in one step by Ni^{2+}-NTA affinity chromatography. Biochemical analysis revealed that the ATP synthase was functional and that the hybrid rotor was also produced in *E. coli*. Deletion of the V_O-type subunit c_1 diminished assembly of the *c* ring and led to a loss of Na^+ transport. These data give the first insights into the structure, function and assembly of a unique membrane rotor of an ATP synthase.

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PHYV024

In search of an electron reservoir that protects the soluble, NAD^+-reducing [NiFe]-hydrogenase from detrimental effects of oxygen.

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The facultative chemolithoautotrophic bacterium *Ralstonia eutropha* H16 is capable of metabolizing H_2 in the presence of O_2 through catalysis of a membrane-bound and a soluble, NAD^+-reducing [NiFe]-hydrogenase (SH). The SH is a bimodular heterohexamer consisiting of five different subunits forming a NAD^+ reductase ($HoxFUI_2$) module and a hydrogenase (HoxHY) module. The hydrogenase module shows homology to standard [NiFe]-hydrogenases with the difference that the SH small subunit HoxY is truncated and contains binding motifs for only one iron-sulfur (FeS) cluster instead of the three FeS clusters arrangement present in standard hydrogenases. Substoichiometric amounts of flavin mononucleotide (FMN) found in the purified HoxHY module in combination with the beneficial effect of externally supplied FMN on the enzyme activity have led to the hypothesis that beside the single FeS cluster, a weakly-bound FMN cofactor is associated with the HoxY subunit [1,2]. While the molecular basis of the high O_2 tolerance of the SH awaits further elucidation, a novel [4Fe-3S] cluster coordinated by six cysteines was recently reported to be involved in the O_2 tolerance of the membrane-bound hydrogenase of *R. eutropha* [3]. This [4Fe-3S] cluster can store two electrons facilitating a fast reductive removal of O_2 as water molecules from the [NiFe] active site in the large subunit of the hydrogenase [4].
To investigate whether a modified FeS cluster and/or an additional FMN cofactor are involved in the O_2 tolerance of the SH, single amino acid exchanges were introduced into HoxY. In total five aromatic amino acids, five cysteine and three histidine residues, conserved only in closely related SH homologues, were exchanged in order to distort the coordination of the FMN and the FeS cluster, respectively.
Four of the resulting mutants show increased O_2 sensitivity upon growth on $H_2/CO_2/O_2$. However, the FMN content of the purified SH variants was not significantly altered compared to the wild-type SH. Strikingly, the exchanged residues W42, W116, C39, C104 are in close proximity to the conserved cysteines C41, C44, C113, C179 proposed to coordinate the FeS cluster according to a structural model. Potential modifications of the FeS cluster in these mutant proteins are currently investigated by electron paramagnetic resonance spectroscopy.

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PHYP001

The electron acceptor tetrathionate supports the growth of *Campylobacter jejuni* under oxygen limitation and is reduced by TsdA, a new type of bi-functional tetrathionate reductase/thiosulphate dehydrogenase widely distributed in bacteria

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Tetrathionate ($S_4O_6^{2-}$) is produced in the vertebrate intestinal mucosa from the oxidation of thiosulphate by reactive oxygen species during inflammation [1], and can be used by some bacteria as an electron acceptor. Surprisingly, the microaerophilic mucosal pathogen Campylobacter jejuni was found to grow under oxygen-limited conditions with tetrathionate, although it does not possess any known type of tetrathionate reductase. Here, we show that in *C. jejuni 81116*, a di-haem cytochrome c (C8j_0815; TsdA) is a bifunctional tetrathionate reductase/thiosulphate dehydrogenase, with a 25-fold higher affinity for tetrathionate compared to thiosulphate. A tsdA mutant could not grow rapidly on tetrathionate under oxygen-limitation, lacked thiosulphate dependent oxygen consumption, failed to convert thiosulphate to tetrathionate in microaerobic cultures, but still slowly reduced tetrathionate, which supported limited growth after an extended lag-period. An additional TsdA homologue (C8j_0040) with an unknown physiological role and which lacks the unusual His-Cys haem ligation of TsdA, nevertheless had low levels of both thiosulphate dehydrogenase and tetrathionate reductase activity. Our data highlight a hitherto unrecognized capacity of *C. jejuni* to use tetrathionate and thiosulphate in its energy metabolism, which we predict is important in the host and reveal a new class of bacterial tetrathionate reductase that is widely distributed amongst bacteria.

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PHYP002

Linalool isomerase, a monoterpene allylalcohol isomerase from *Thauera linaloolentis* 47Lol^T

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Monoterpenes and their oxygenated derivatives are produced and emitted by plants. In the atmosphere, monoterpenes are vulnerable to photochemical oxidation, whereas microorganisms metabolize those compounds under aerobic and anaerobic conditions. In contrast to the aerobic metabolism, so far only little is known about the anaerobic metabolism of monoterpenes.
The Gram-negative *Betaproteobacterium Thauera linaloolentis* 47Lol^T is able to use monoterpenoids as sole energy and carbon source. To our current knowledge, it grows on (R,S)-linalool and geraniol under denitrifying conditions.
The isomerization of the tertiary alcohol linalool to the primary alcohol geraniol is catalyzed by a linalool isomerase (LI). This reaction proceeds without the formation of nerol, the *cis*-isomere of geraniol. Linalool isomerase activity has been found in cell cultures as well as in cell free protein extracts. The isomerization is similar to the isomerase function of the linalool dehydratase-isomerase (LDI) from *C. defragrans* 65Phen. The LDI catalyses stereospecifically the formation of (S)-(+)-linalool from myrcene and its further isomerization to geraniol.
The poster will present a developed enzyme assay, first attempts on the purification of the linalool isomerase and study results of the catalyzed reaction.

PHYP003

The role of *Desulfitobacterium spp.* in the global network of *O*-demethylation in soil

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Desulfitobacterium spp. are strictly anaerobic bacteria first isolated from environments contaminated with halogenated compounds. It is known that at least two strains of *Desulfitobacterium hafniense* (DCB-2 and PCE-S) are able to use phenyl methyl ethers, which are natural degradation products of

lignin, as electron donors. By then, only acetogens had been reported to convert these compounds under anoxic conditions. In contrast to acetogenic bacteria, *D. hafniense* is not able to use CO_2 as electron acceptor. For this reason, appropriate electron acceptors have to be provided by the soil microbial community. So far, it is not known what the physiological electron acceptors in natural environments, such as forest soils, are and which organisms provide these compounds.
To study the impact of Desulfitobacteria on the *O*-demethylation process in soil, several *Desulfitobacterium* strains were tested for their ability to consume phenyl methyl ethers in the presence of fumarate as electron acceptor. Furthermore, nitrate, sulfite, and thiosulfate were tested as potential physiological electron acceptors in soil. First results indicate that *O*-demethylation seems to be a common feature of *D. dehalogenans* and different *D. hafniense* strains. The screening of genomes of different Desulfitobacteria revealed the presence of a high number of putative *O*-demethylase operons. For *D. hafniense* DCB-2, the genes of one of the predicted operons were cloned and expressed in *Escherichia coli*. The characterization of the corresponding gene products led to the first isolation of a functional *O*-demethylase which originates from Desulfiobacteria.

PHYP004
Purification and characterization of an NAD^+-dependent XylB-like aryl alcohol dehydrogenase identified in *Acineto-bacter baylyi* ADP1

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Reports on alcohol dehydrogenases in *Acinetobacter* strains are quite rare. Until now AlrA and AreB were the only described alcohol dehydrogenases in this strain. Herein, we report the purification and characterization of a XylB-like alcohol dehydrogenase from *A. baylyi* ADP1 (Uthoff and Steinbüchel, 2012). The gene $xylB_{ADP1}$ from *A. baylyi* ADP1 (ACIAD1578) coding for a putative aryl alcohol dehydrogenase was heterologously expressed in *Escherichia coli* BL21 (DE3). The respective enzyme was purified by fast protein liquid chromatography to apparent electrophoretic homogeneity. The predicted molecular size of 39,500 Da per subunit was confirmed by SDS polyacrylamide gel electrophoresis. According to the native M_w as determined by gel filtration, the enzyme forms dimers indicating a relationship to XylB enzymes of e.g. *Pseudomonas putida*. The enzyme was NADH-dependent and able to reduce medium to long chain length *n*-alkylaldehydes, methyl branched aldehydes and aromatic aldehydes with benzaldehyde yielding the highest activity. The oxidation reaction with the corresponding alcohols showed only 2.2% of the reduction activity with coniferyl alcohol yielding the highest activity. Maximum activities for the reduction and the oxidation reaction were 104.5 and 2.3 U mg^{-1} of protein, respectively. The enzyme activity was affected by low concentrations of Ag^+ and Hg^{2+} and high concentrations of Cu^{2+}, Zn^{2+} and Fe^{2+}. The gene $xylB_{ADP1}$ seems to be expressed constitutively and an involvement in coniferyl alcohol degradation is suggested.

Literature:
Uthoff S. and Steinbüchel A. 2012. Purification and characterization of an NAD^+-dependent XylB-like aryl alcohol dehydrogenase identified in *Acinetobacter baylyi* ADP1. Appl Environ Microbiol. 2012 October 5 [Epub ahead of print]

PHYP005
Microbial gutta percha degradation shares common steps with rubber degradation in *Nocardia nova* SH22a

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Gutta percha (GP) and natural rubber (NR), consisting of the *trans*- and *cis*-isomers of isoprene, respectively, are important basic materials in our life. The increasing production of GP and NR products led to an accretive problem of waste treatment. About biodegradation of GP almost nothing is known in contrast to the extensively studied degradation of NR (1). *Nocardia nova* SH22a, a bacterium capable of degrading GP and NR, was employed in this study to investigate the GP degradation mechanism and relations between the GP and NR degradation pathways (2). For this strain, a protocol of electroporation was systematically optimized, and an efficiency of up to 4.3×10^7 colony forming units per µg plasmid DNA was achieved. By applying this optimized protocol to *N. nova* SH22a, a Tn*5096*-based transposon mutagenesis library of this bacterium was constructed. Among about 12,000 apramycin resistant transformants, we identified 76 stable mutants defective in GP or NR utilization. Whereas 10 mutants were specifically defective in GP utilization, the growth of the other 66 mutants was affected on both GP and NR. This indicated that the two degradation pathways are quite similar and share many common steps. The larger number of GP-degrading defective mutants could be explained as either (i) the GP pathway is more complex and harbors more specific steps, or (ii) the steps for both pathways are almost identical, but in case of GP degradation there are less enzymes involved in each step. The analysis of transposition loci and genetic studies on interesting genes confirmed the crucial role of α-methylacyl-CoA racemase in the degradation of both GP and NR. This study also demonstrated the probable implication of enzymes, which are involved in oxidoreduction reactions, β-oxidation and the synthesis of cell envelope in the degradation of GP.

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PHYP006
Mrp1 and Mrp2 are involved in cation/proton antiport in *Corynebacterium glutamicum*

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Besides H^+, Na^+ and K^+ ions are the most import cations for bacterial cells. Sodium ions are not only used for sodium-coupled energy conservation and energy transduction but also for solute uptake, pH homeostasis and activation of intracellular enzymes [3]. Potassium represents the most abundant cation in the prokaryotic cytoplasm and plays a role in the control of the membrane potential, regulation of the internal pH value, activation of enzymes and osmotic stress response [1,2,4,5]. To investigate ion homeostasis it is necessary to know the transport systems involved in import and export.
In *Corynebacterium glutamicum* Na^+ ions are mainly taken up by Na^+/solute symport. For K^+ ions there is only one functional uptake system present, the channel CglK [5]. The export of Na^+ and K^+ has not been investigated so far. There are four putative cation/proton antiporters encoded in the genome: ChaA, NhaP, Mrp1, Mrp2. The latter two transporters belong to the CPA-3 (cation:proton antiporter-3) class that comprises multiple resistance and pH-related antiporter systems (mrp) [6]. The encoding genes are organized in an operon and their products form hetero-oligomeric complexes in the membrane. Their function in Na^+, K^+, and high pH resistance have been ascribed to their activity as $Na^+(Li^+)/H^+$ and/or K^+/H^+ antiporters.
Each of *C.glutamicum*'s *mrp* operons comprises six genes: *cgl0269-cgl0264* (*mrp1*) and *cgl2729-cgl2734* (*mrp2*). The physiological characterization of Mrp1 and Mrp2 in *C.glutamicum* revealed roles in Na^+/H^+- and K^+/H^+-antiport, respectively, as well as pH homeostasis at alkaline pH. The possible contribution of the additional systems ChaA and NhaP was also investigated.

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PHYP007
The effect of CO_2 on pH homeostasis and an alternative way for inorganic carbon provision in *Corynebacterium glutamicum*

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The Gram-positive soil bacterium *C. glutamicum* is extensively used in industrial lysine production. For gluconeogenesis as well as for lysine production, carboxylation of pyruvate and phosphoenol pyruvate (PEP) are key reactions which require bicarbonate (HCO_3^-) as substrate. Its provision is ensured by hydration of CO_2. This reaction is catalysed by the ß-type carbonic anhydrase Bca and leads to the formation of carbonic acid which dissociates into bicarbonate and a proton[1]. Thus, on the one hand CO_2 might have a negative influence on pH homeostasis under acidic conditions due to the proton formation. On the other hand, essentiality of inorganic carbon

from CO_2 is reflected in the growth deficit of the Δ*bca* mutant at an atmospheric CO_2 concentration[1]. We investigated the impact of high CO_2 concentrations on the pH homoeostasis of liquid cultures online using a pH sensitive fluorescence dye[2]. Upon acidification of the outer medium, the intracellular pH (pH_i) drops to a lower value if cells are exposed to 20 % CO_2, although the same steady-state pH_i is reached compared to atmospheric CO_2 exposure. To investigate a way of provision with inorganic carbon other than hydration of CO_2, we heterologously expressed the HCO_3^- importer SbtAB from *Synechocystis spec*. PCC 6803 in *C. glutamicum*. The transport protein is active and able to restore growth of the Δ*bca* strain at atmospheric CO_2 concentrations on various organic carbon sources by providing sufficient amounts of inorganic carbon. This observation makes SbtAB a promising tool for optimisation of inorganic carbon supply in industrially used lysine producing strains of *C. glutamicum*.

1) Mitsuhashi et al., 2004
2) Miesenboeck et al., 1998

PHYP008

The dual function of the MscCG channel from *Corynebacterium glutamicum*

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Corynebacterium glutamicum is a Gram-positive, biotin auxotroph and apathogenic soil bacterium with exceptional importance for the industrial production of various amino acids, especially L-glutamate. The mechanism of glutamate export is not fully understood so far, although *C. glutamicum* has been used for the industrial production of amino acids for decades. Glutamate excretion can be induced by several different treatments, all altering the cell envelope. Recently, evidence was provided that the channel MscCG of *C. glutamicum* is linked to glutamate excretion under glutamate production conditions. MscCG belongs to the MscS-type family of mechanosensitive channels, functioning as emergency valves upon an osmotic downshift. The protein is a close relative of the mechanosensitive channel MscS from *E. coli* (286 AA) concerning its N-terminal and pore region. In addition, MscCG carries a long C-terminal domain of 247 amino acids including a fourth transmembrane domain. The electrophysiological analysis of MscCG showed the typical pressure dependent gating behavior of a stretch-activated channel with a current/voltage dependence indicating a strongly rectifying behavior. To unravel the dual function of MscCG as a mechanosensitive channel and as a glutamate exporter, the well characterized *E. coli* homolog of MscCG, MscS, was used and expressed in a Δ*mscCG* strain. We also generated selected C-terminal truncations of MscCG in *C. glutamicum*, gain-of-function and loss-of-function constructs of *C. glutamicum* MscCG, as well as fusion constructs of MscS and MscCG, and we have investigated the properties of these constructs with respect to mechanosensitive efflux, electrical conductance, gating properties, as well as glutamate excretion. Various recombinant forms of MscCG were shown to be closely similar with respect to conductance, but we found significant differences concerning glutamate excretion. The results of these experiments argue for MscCG being both a relevant mechanosensitive channel in *C. glutamicum* upon hypoosmotic stress as well as the major efflux pathway for glutamate excretion in response to particular physiologic conditions. Moreover, the results obtained indicate the C-terminal domain of MscCG being of significant impact for function and/or regulation of MscCG activity.

PHYP009

Characterization of PTS mediated fructose uptake in *Corynebacterium glutamicum*

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Corynebacterium glutamicum is employed for the large-scale production of amino acids such as L-glutamate and L-Lysine. For industrial fermentations beside glucose and sucrose also fructose is used as feedstock. Uptake and phosphorylation of these sugars are brought about via the PEP: carbohydrate phosphotransferase system (PTS), which consists of the general components EI and HPr and four substrate-specific EII permeases. Fructose is taken up and phosphorylated to fructose-1-phosphate by the *ptsF* encoded EII_{Fru}. Fructose-1-phophate is then metabolized via fructose-1-phosphate kinase to the glycolysis intermediate fructose-1,6-bisphosphate. Overproduction of L-lysine comes along with a high NADPH demand, which is covered mainly by the pentose phosphate pathway (PPP). Metabolic flux studies of *C. glutamicum* cultured on fructose indicated, that the glucose-6-phosphate required for the PPP is not formed from fructose-1,6-bisphosphate, as no activity of fructose-1,6-bisphosphatase was observed [1]. Instead, it was supposed that a minor part of fructose is taken up and phosphorylated to fructose-6-phosphate by the *ptsG* encoded EII_{Gluc} and then isomerized to glucose-6-phosphate by the phosphoglucoisomerase.
We here characterised fructose uptake using ^{14}C-labelled fructose in the *ptsF* deletion mutant *C. glutamicum* Δ*ptsF,* which proceeded with a reduced rate and lowered affinity (K_M 1016 μM, V_{max} 9 nmol/(min*mg CDW)) than fructose uptake in *C. glutamicum* WT (K_M 52 μM, V_{max} 70 nmol/(min*mg CDW)). As both growth on and uptake of fructose were absent in the double mutant *C. glutamicum* Δ*ptsF*Δ*ptsG,* we concluded that the *ptsG* encoded EII_{Glc} is indeed responsible for the EII_{Fru}-independent fructose uptake in *C. glutamicum*. Northern blot analyses and reporter gene assays showed *ptsG* less expressed in *C. glutamicum* during cultivation on fructose than on glucose. Furthermore EII_{Gluc}-specific uptake of ^{14}C-labelled glucose was also slower in *C. glutamicum* cells from cultivations on fructose compared to cells cultivated on glucose. Taken together, overexpression of *ptsG* in *C. glutamicum* might be beneficial for L-Lysine production on fructose, as by this means the EII_{Glc} mediated fructose-6-P formation and thereby flux through the PPP might be improved.

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PHYP010

Metabolic engineering of microorganisms for the production of stilbenes

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Currently, access to plant natural products is based either on the extraction from plants or on chemical synthesis. These approaches are often expensive and therefore commercially not feasible. Efficient heterologous synthesis of such plant-derived substances in bacterial hosts is therefore of great interest, especially when larger quantities are required for pharmacological or other applications.
Stilbenes are phenylpropanoid-derived compounds with a 1,2-diphenylethylene backbone that can be found in a wide variety of plants [1]. The stilbene pinosylvin protects the plants against viral and microbial attack and UV exposure [2]. Several studies also indicate its potential benefits on human health in the treatment of various diseases such as cancer [3] and arthritis [4].
We focus on the heterologous production of pinosylvin in two well-known industrial production hosts, *Escherichia coli* and *Corynebacterium glutamicum*. Biosynthesis of pinosylvin is achieved in three enzymatic steps from the proteinogenic amino acid L-phenylalanine.
Based on their reported kinetic parameters and already proven functional expression in *E. coli*, two to three candidate enzymes for each enzymatic step were selected. Subsequently, genes were designed for an optimal heterologous expression and chemically synthesized. First experiments in which the genes of the pinosylvin pathway were systematically combined and expressed in *E. coli* yielded strains producing up to 350 mg/L *trans*-cinnamic acid levels in the supernatant, whereas only low amounts of pinosylvin could be detected by LC-MS. Current efforts focus on the identification of metabolic bottlenecks and the optimization of cultivation and production conditions.

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PHYP011

Corynebacterium glutamicum harbours a molybdenum cofactor-dependent for-mate dehydrogenase which alleviates growth inhibition in the presence of formate

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We show that *Corynebacterium glutamicum* ATCC 13032 co-metabolizes formate when it is grown with glucose as the carbon and energy source. CO_2 measurements during bioreactor cultivation and use of ^{13}C-labelled formate

demonstrated that formate is almost completely oxidized to CO_2. The deletion of *fdhF* (*cg0618*), annotated as formate dehydrogenase (FDH) and located in a cluster of genes conserved in the family *Corynebacteriaceae*, prevented formate utilization. Similarly, deletion of *fdhD* (*cg0616*) resulted in the inability to metabolize formate and deletion of *cg0617* markedly reduced formate utilization. These results illustrated that all three gene products are required for FDH activity. Growth studies with molybdate and tungstate indicated that the FDH from *C. glutamicum* ATCC 13032 is a molybdenum-dependent enzyme. The presence of 100 mM formate caused a 25% lowered growth rate during cultivation of *C. glutamicum* ATCC 13032 wild-type in glucose minimal medium. This inhibitory effect was increased in the strains lacking FDH activity. Our data demonstrate that *C. glutamicum* ATCC 13032 possesses an FDH with a currently unknown electron acceptor. The presence of the FDH might help this soil bacterium to alleviate growth retardation caused by formate, which is ubiquitously present in the environment.

PHYP012

Stress-induced activation of a cryptic gene in *Bacillus subtilis* by adaptive mutagenesis

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Bacteria can cope with many growth conditions by adjusting gene expression and metabolic pathways. Beside these transient responses, bacteria can spontaneously accumulate mutations or shape their genomes in response to stress (1,2,3,4). The latter evolutionary scenario is a matter of controversy as it implies the existence of stress-sensing factors that modify the chromosome (5). Recently a *bona fide* Lamarckian phenomenon has been identified in the model bacterium *Bacillus subtilis* (6). A mutant deficient for an active glutamate dehydrogenase (GDH) rapidly activates the cryptic, GDH encoding $gudB^{CR}$ gene by deleting 9 bp of a direct repeat (DR) present in the gene, and the resulting suppressors synthesise the active GDH GudB. Competition experiments revealed that it is beneficial for the bacteria to express two functional GDHs with excess nitrogen; by contrast, keeping the $gudB^{CR}$ allele provides a selective advantage when nitrogen is limiting. Moreover, we found that under nitrogen-limiting conditions the $gudB^{CR}$ allele is rather stable while in the presence of glutamate $gudB^{CR}$ is rapidly converted to the active *gudB* allele. Thus, the availability of nitrogen is a driving force for the activation and inactivation of GDH encoding genes in *Bacillus subtilis*. By following the emergence of suppressor mutants over time we found that $gudB^{CR}$ was modified while other genes containing perfect DRs remained unaffected. Even more astonishing, the mutagenesis apparatus is obviously able to anticipate the selective advantage provided by the mutation: the mutation occurred when the resulting *gudB* allele gave rise to an active enzyme. In contrast, a $gudB^{CR}$ variant that could be mutated without encoding an active protein was not mutated. As the highly specific mutagenic event resembles the Lamarckian form of evolution we expect our system to be a starting point for the identification of factors that sense stress and enable the cell to respond at the genome level.

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PHYP013

Membrane lipid composition regulates activity and localization of *Agrobacterium tumefaciens* phospholipid *N*-methyltransferase PmtA

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The phospholipid *N*-methyltransferase PmtA is one of the key enzymes responsible for phosphatidylcholine (PC) synthesis in the plant pathogen *Agrobacterium tumefaciens*. The typical eukaryotic phospholipid PC is a main component of *A. tumefaciens* membranes and is crucial for tumor formation [1, 3]. PC production via PmtA follows a sequential *N*-methylation of the membrane component phosphatidylethanolamine (PE) via the intermediates monomethyl-PE and dimethyl-PE. *S*-adenosylmethionine (SAM) is used as methyl donor and is converted to *S*-adenosylhomocysteine (SAH) in this reaction [2, 4]. The interaction of PmtA with cell membrane seems to be reversible since PmtA can be purified from cytosolic fractions [2, 3].

Here, we investigated the regulatory mechanisms controlling the membrane affinity of PmtA. We produced PmtA in *E. coli* and purified the protein for *in vitro* characterization of membrane binding properties. Analysis of PmtA *in vitro* activity revealed a stimulatory effect of anionic lipids. Liposome co-sedimentation assays demonstrated a tight binding of PmtA to liposomes containing anionic lipids. This interaction of PmtA with anionic lipids is driven by non-specific electrostatic forces since association to liposomes with anionic lipids is abolished under high ionic strength conditions. PmtA binding to liposomes with increasing PC content is significantly decreased suggesting a negative regulatory effect of the end product on membrane association. Taken together, our results provide strong evidence that membrane to cytosol cycling of PmtA is regulated by the membrane lipid composition.

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PHYP014

Investigation of the scale-up related CO_2/HCO_3^--stimulus in *Corynebacterium glutamicum*

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Corynebacterium glutamicum is a facultative anaerobic, Gram-positive organism that grows on a variety of sugars and organic acids and is the workhorse for the large scale production of a number of amino acids, such as L-glutamate and L-lysine. In large scale production processes typical bioreactor volumes reach up to 750 m^3 to keep production costs low, resulting in mixing times up to 240 s (Kelle et al. 2005; Junker, 2004). Consequently, cells are exposed to gradients of temperature, substrate concentrations, pH-values or partial pressures of the various diluted gases as e.g. O_2 and CO_2 (Lara et al., 2006). The latter is additionally enforced due to the hydrostatic pressure, caused by the reactor height and the existence of poorly ventilated regions due to larger volumes (Baez et al., 2011). Here, we investigated the scale-up related CO_2/HCO_3^--stimulus on the metabolism of *Corynebacterium glutamicum*. Batch-fermentations with glucose as sole carbon and energy source with elevated CO_2 levels of up to 30 % in the inlet gas flow showed no difference in the growth rate but slightly higher biomass yield compared to standard conditions indicating that *C. glutamicum* can tolerate high CO_2/HCO_3^--concentrations. In contrast, experiments with significantly reduced CO_2 concentrations obtained by high aeration (3 vvm) with air led to diauxic-like growth, which can be complemented by increasing the proportion of CO_2 in the inlet gas flow. Transcriptome profiles obtained by microarray analysis in combination with determination of enzyme activities led to a comprehensive picture of the adaptation to CO_2/HCO_3^--limiting conditions, indicating an indirect activation of decarboxylation reactions, whereas high CO_2/HCO_3^- conditions probably result in oxidative stress response.

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PHYP015

The influence of an additional NADH Dehydrogenase on the growth behaviour in *G. oxydans* DSM3504

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The genus *Gluconobacter* from the family of Acetobacteriaceae is well known for its ability to perform rapid incomplete oxidations of a great variety of carbohydrates, alcohols and related compounds. In a multitude of biotechnological processes *G. oxydans* is used because of itsregio- and stereo-selective oxidative capabilities. Due to its biotechnological relevance the investigation of growth characteristics of different strains is of major importance. In order to increase future growth yields of industrial strains we investigated the genomes of the strains G. oxydans 621H und DSM3504.
G. oxydans 621H is an industrial production strain that was selected for its high substrate oxidation rates. This strain shows low growth yields, which is impeding its applications. The recently sequenced strain *G. oxydans* DSM3504 however exhibits a high similarity to strain 621H on genomic level, but the growth yield is about three times higher than in strain 621H. The sequence based comparison of the two strains revealed that several genes are present in DSM3504 but lacking in 621H. We created different clean deletion mutants of DSM3504 to investigate their effect on growth yield. Among others strain DSM3504 contains an additional NADH-dehydrogenase. Therefore, a mutant lacking this enzyme was constructed and characterized. Growth experiments were performed with RAMOS (Respiration Activity MOnitoring System) and Biolector. These systems allowed measurements of scattered light (biomass), NADH- and FAD concentration, O_2 and CO_2 transfer rates. The evaluation of these data together with growth studies confirmed the important role of the additional NADH-dehydrogenase for the growth behavior. These results were confirmed by expression of the NADH gene in strain 621H, which leads to a significant increase of the growth rate.

PHYP016

Reductive activation of protein bound vitamin B_{12} - characterization of a new metallo-ATPase by site directed mutagenesis

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Coupling unfavorable electron transfer to ATP hydrolysis is fundamental in the field of bioenergetics. Three classes of electron transferring, metal-containing ATPases are known. They couple exergonic ATP hydrolysis to electron transfer from a moderate potential active site to a low potential site. One class of metallo-ATPases comprises the dinitrogen fixing nitrogenases (Seefeldt *et al*., 2009). A second class are the radical forming β,α-dehydratase-like proteins (Boll & Fuchs, 1995; Buckel *et al*., 2004,). The third class was just recently discovered. These enzymes are associated with cobalamin dependent methyltransferase systems and were therefore referred to as RACEs (reductive activator of corrinoid-dependent enzyme). Cob(I)alamin is sensitive to autooxidation and needs occasional reactivation which is mediated by this class of metallo-ATPases. Until now, three different RACEs were studied in more detail: RamA involved in the methylamine methyltransferase reaction of *Methanosarcina barkeri* (Ferguson *et al*., 2009), RACo as reductive activator of the corrinoid/iron-sulfur protein of *Carboxydothermus hydrogenoformans* (Hennig *et al*., 2012) and the activating enzyme (AE) as part of the *O*-demethylases of *Acetobacterium dehalogenans* (Schilhabel, Studenik *et al*., 2009). Studies on the activation mechanism of *O*-demethylases are subject of the work presented here.
Different mutants of AE were produced by exchange of a single amino acid within a small conserved region found in RACEs. These mutants were studied with respect to their ability of ATP hydrolysis, corrinoid reduction and complex formation with the corrinoid containing protein.
The interpretation of the results was assisted by the prediction of a reliable three-dimensional protein structure derived from the recently published RACE structure of RACo (Hennig *et al*., 2012).

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PHYP017

Characterization of two *Staphylococcus equorum yoeB-yefM* Toxin-Antitoxin System Homologues

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Toxin-Antitoxin (TA) systems are bicistronic genetic elements that consist of a stabile proteic toxin and an unstable RNA or protein antitoxin. Their functions in prokaryotes include plasmid stabilization, adaptation to stress and formation of persister cells. A homology based *in silico* screen in the non-pathogenic organism *Staphylococcus equorum*, revealed ten putative type TA loci. A *mazEF* TA locus encoding a type II TA system (with both toxin and antitoxin as proteins) was characterized previously [1]. In *S. aureus* the YoeB toxins are ribosome dependent RNases and we here analyzed two paralogous *yefM-yoeB* loci of *S. equorum*. Transcriptional start sites were mapped by 5' RACE, which concomitantly confirmed their expression in the exponential growth phase. The putative toxin $YoeB_{1/2}$ encoding genes were cloned into the arabinose inducible vector pBAD33 and their cognate antitoxins $YefM_{1/2}$ in the IPTG inducible vector pET21c. Toxin expression lead to a growth defect in *E. coli* BL21(DE3), which could be counteracted by concomitant expression of the cognate antitoxins $yefM_{1/2}$. Interestingly, the antitoxins could not cross-suppress toxicity of the respective paralogous toxin. These results indicate that the two *yefM-yoeB* paralogues encode functional TA systems of *S. equorum*. Further characterization includes *in vivo* interaction analyses of the toxin and antitoxin by a bacterial two hybrid system. We would also like to analyze YoeB toxins regarding RNase activity and critical amino acid positions by random or site-directed mutagenesis.

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PHYP018

Cell wall biosynthesis reactions in *Staphylococcus aureus* are positively influenced by anionic phospholipids

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Phospholipids are major constituents of biological membranes. They play an important role in many fundamental cellular processes in prokaryotes and eukaryotes. Membrane-associated proteins and integral membrane proteins are attached and inserted into the membrane according to the prevalent lipid environment. Furthermore, a specific interaction of proteins with certain lipid species was described for several proteins involved in respiration, photosynthesis, solute transport, motility and signal transduction. Tight binding of lipids increases structural stability, facilitates oligomerization processes and improves folding and insertion, thereby controlling functional activity. It is conceivable that phospholipids have an impact on cell wall biosynthesis reactions in prokaryotes - especially those reactions using the membrane-standing carrier $C_{55}P$ or $C_{55}P$-containing precursors as a substrate.
We show here that TarO and MraY, glycosyltransferases catalyzing the first committed step in wall teichoic acid and peptidoglycan biosynthesis, as well as the phosphatase UppP are positively influenced by anionic phospholipids (phosphatidylglycerol, cardiolipin) in vitro. The impact of various lipid species on activity modulation of the putative WTA-transferase MsrR will be investigated by use of the full-length protein as well as shortened variants.

PHYP019

Protein complexes in the respiratory chain of *Dehalococcoides mccartyi* CBDB1

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The genus *Dehalococcoides* belongs to the phylum *Choroflexi* and comprises strictly anaerobic bacteria that conserve energy exclusively by organohalide respiration. *Dehalococcoides* species reductively dechlorinate a variety of halogenated organic compounds using H_2 as the sole electron donor and acetate and CO_2 as carbon sources. Beside 32 homologous genes coding for cobalt-dependent reductive dehalogenases (Rdh), the genome of *D. mccartyi* CBDB1 also encodes 5 different types of multisubunit hydrogenases, which further emphasizes the specialization of *D. mccartyi* CBDB1 for this mode of respiration. However, the biochemistry of organohalide respiration is still poorly understood and it is the aim of this project to provide information of the mechanisms underlying this energy-conserving process. In an attempt to characterize the role of the two enzymes in organohalide respiration in more detail, we first detected hydrogenase and dehalogenase activity in native PAGE gels using crude extracts of *D. mccartyi* CBDB1. Then, cross-linking experiments were performed to study protein-protein interactions between hydrogenase, reductive dehalogenases and other possible components of the respiratory chain and a large characteristic complex was observed. Additionally, cross-linking experiments provide evidence that the hydrogenase encoded by the hup gene might be associated with the Rdh. Hup represents the only hydrogenase complex for which the active site is predicted to be located on the extracellular side of the cytoplasmic membrane and is therefore of special interest for H_2 oxidation during energy conservation. Furthermore, expression studies of the genes encoding the catalytic subunits of the hydrogenases were performed using qRT-PCR for cultures grown in the presence or absence of electron donor and acceptor, respectively. The results show that these genes were highly expressed under each condition tested. For the dehalogenases, a correlation between synthesis of particular reductive dehalogenases and cultivation on different halogenated electron acceptors was observed using enzyme activity assays and mass spectrometric analysis. This indicates the involvement of a series of Rdh proteins in various dehalogenation reactions. Acknowledgement: This work is supported by the DFG (Research Unit FOR1530).

PHYP020

Towards the identification of the amino acid determinants in FocA important for formate translocation in *E. coli*

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During mixed acid fermentation enterobacteria such as *E. coli* are able to convert one third of the carbon derived from glucose to formate. The accumulation of formate in the *E. coli* cell can, however, lead to acidification of the cytoplasm and therefore mechanisms to regulate its level must be available. *E. coli* possesses three different formate dehydrogenases (FDH). One of them is located in the cytoplasm and forms together with hydrogenase 3 the formate hydrogenlyase (FHL) complex. The other two FDHs have their catalytic site in the periplasm. Therefore, intracellularly generated formate has to be transported across the cytoplasmic membrane. This translocation is performed by FocA, which was identified as a formate channel[1]. FocA is a homopentamer that functions in a bidirectional manner and belongs to the superfamily of formate-nitrite transporters (FNT). Although several structures have been published recently[2], there is still no clear mechanistic understanding of how formate passage though the FocA proteins is controlled. Nevertheless, the structural analysis of FocA identified key amino acids that are likely involved in channelling formate through the protein. It appears clear that formate is translocated through each of the monomeric pores of FocA. Therefore conserved amino acids in the pore region were exchanged to examine the consequences on formate translocation. Many of these amino acids are highly conserved within the family and might be important in channel 'gating'. We could show that single amino acid exchanges of T91A, H209Y and N213D influenced formate transport significantly and affected directionality of formate movement through FocA.

Furthermore we could demonstrate that formate translocation and formate metabolism are inextricably linked.

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PHYP021

The Influence of Carbon Dioxide on the Maturation and Activity of the [NiFe]-Hydrogenases in *Escherichia coli*

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Anaerobically grown *Escherichia coli* synthesizes three membrane-bound [NiFe]-hydrogenases (Hyd). Hyd-1 and Hyd-2 oxidize molecular hydrogen whereas Hyd-3 evolves hydrogen and forms part of the formate hydrogenlyase (FHL) complex. The active site contains a Ni atom coordinated by four cysteine thiolates. Two of the cysteine side chains coordinate additionally the iron, which has two CN^- ligands and one CO ligand attached. The metabolic origin of the CN^- ligands is carbamoylphosphate whereas the source of the carbon monoxide ligand is still unknown. It has been proposed that locally produced CO_2 could be the source of the CO[1]. If endogenous CO_2 is the precursor, this would require a reductase to reduce CO2. One possible candidate is the FeS enzyme HypD, which has been proposed to function late in the maturation pathway. We could show that anaerobically isolated HypD-HypC complexes indeed carry CN^-, CO and CO_2 supporting the proposal that CO_2 might be the precursor of CO. An important enzyme in CO_2 metabolism is carbonic anhydrase, which catalyzes the reversible hydration of CO2 to bicarbonate[2]. To analyse the potential importance of CO2 for hydrogen metabolism in *E. coli* we constructed conditional *can* mutants and examined the effect of exogenous CO_2 on growth in minimal media and on hydrogenase synthesis and maturation. We monitored enzyme activities and used western blotting to analyze the maturation of the large subunits of all three hydrogenases. Our findings show a clear link between CO_2 and H_2 metabolism.

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PHYP022

Involvement of a putative formylmethanofuran dehydrogenase during carbon monoxide-dependent growth of *Methanosarcina acetivorans*

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Methanosarcina acetivorans, a marine methanogenic archaeon, is able to utilize methylated compounds, acetate, and carbon monoxide (CO) as sole source of energy for growth but not H_2+CO_2. During CO-dependent growth the organism produces, beside methane, substantial amounts of acetate and formate. Formyl-methanofuran dehydrogenases (FMD) are multi-subunit molybdenum or tungsten iron-sulfur proteins, which use methanofuran as co-substrate, and catalyze the ferredoxin-dependent reduction of carbon dioxide to formyl-methanofuran, the initial reaction of CO_2 reduction to methane. The genome of *M. acetivorans* encodes four putative FMD homologs, two of the molybdenum type (here termed Fmd1 and Fmd2) and two of the tungsten type (termed Fwd1 and Fwd2). Comparative gene analyses indicate that Fwd1 and Fwd2 lack the gene for a polyferredoxin thought to be involved in electron transfer. Reporter gene fusion analysis revealed a moderate (three-fold) but CO-specific increase of *fwd1* expression during growth. Surprisingly, deletion of *fwd1* severely impaired the organism's ability to grow on CO, while growth on other substrates was not affected. The growth phenotype and the dramatically affected rates of metabolite formation in the *fwd1* mutant indicate a key role of Fwd1 during reduction of CO to methane and argue for distinct functions for the different FMD isoforms in *M. acetivorans*.

PHYP023

Nitrous oxide turnover in the nitrate-ammonifying Epsilonproteobacterium *Wolinella succinogenes*

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Global warming is moving more and more to the public consciousness. Besides the commonly mentioned carbon dioxide and methane, nitrous oxide (N_2O) is one of the most important greenhouse gases and accounts for about 10% of the anthropogenic greenhouse effect.
Several microbial energy converting processes appear to be the most important sources of N_2O emission and the responsible organisms comprise denitrifiers and ammonifiers of nitrate as well as nitrifying bacteria and archaea. Interestingly, some respiratory nitrate-ammonifying Epsilonproteobacteria are able to reduce nitrous oxide to dinitrogen via an unconventional cytochrome *c* nitrous oxide reductase (*c*NosZ). The energy metabolism of one of these bacteria, *Wolinella succinogenes*, has been characterized thoroughly in the past. The cells are able to use formate or hydrogen gas as electron donors together with typical terminal electron acceptors like, for example, fumarate, polysulfide, nitrate, nitrite or nitrous oxide.
Despite the presence of *c*NosZ there is neither a typical nitric oxide-producing nitrite reductase (NirS, NirK) nor a nitric oxide reductase of the Nor-type present in *W. succinogenes*. It is speculated that N_2O might be produced during nitrite and/or NO detoxification by the flavodiiron protein Fdp. Using different ratios of the electron acceptor nitrate and the donor formate the production and consumption of nitrous oxide during nitrate respiration of *W. succinogenes* and various mutant strains were characterized. Furthermore, a model of the electron transport chain of *W. succinogenes* nitrous oxide respiration will be presented.

PHYP024

Modulation of *Pseudomonas* sp. strain VLB120ΔC biofilms

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Biofilms are ubiquitous associated communities of microorganisms frequently embedded in an extracellular polymeric matrix (EPS). Due to their great impact on industrial, medical and environmental settings, there is an increasing interest in understanding and controlling biofilm formation. In recent studies a lot of biofilm related genes and genomic regulation systems could be identified. Nevertheless, the complex molecular network of biofilm development is poorly understood. A key regulator of biofilm formation and dispersion is the ubiquitous intracellular second messenger cyclic-di-GMP (c-di-GMP) [1]. The signal molecule is synthesized by diguanylate cyclases (DGCs), which carry the highly conserved amino acid motive GGDEF, and degraded by phosphodiesterases (PDEs) carrying an EAL domain. Our model strain *Pseudomonas* sp. strain VLB120ΔC [2] encodes a large number of enzymes containing GGDEF- or/and EAL domains. To investigate the impact of c-di-GMP on biofilm formation, *Pseudomonas* sp. strain VLB120ΔC deletion mutants of over 40 DGC and PDE genes were generated via an efficient markerless gene knockout method, which also allows the combination of single knock-out effects in a multiple deletion mutant.
Subsequently, the growth and attachment behavior of the resulting mutants were analyzed and compared to the respective wild type strain. For that reason a novel biofilm formation assay was established. It is based on a tubular cultivation method based on a recently published tubular biofilm reactor system [3] and combines phenotypic analyses of biofilm colonies and direct biofilm quantification. By this highly sensitive, fast and reproducible method the impact of the respective knock-out mutations on the initial biofilm formation rate was investigated. Details on the screening method as well as the outcome of the trial will be presented.

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PHYP025

Effect of environmental stressors on the degradative and adaptive capacities of *Arthrobacter chlorophenolicus* A6 in different formulations

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Bioremediation of sites contaminated with organic pollutants by the bioaugmentation technology is often limited among others by the application of the chosen organism to the contaminated site. Formulated microorganisms provide easy storage, transport and dispersal and therefore are essential for functional and economically sound bioaugmentation. Previous studies have shown, that the psychrophilic Gram-positive bacterium *Arthrobacter chlorophenolicus* A6 is able to degrade unusual high concentrations of 4-chlorophenol, can cope with fluctuating and low temperatures and is tolerant towards high salt concentrations, as well. In this study, we compared the performance of fresh and dried cells of this bacterium during 4-chlorophenol degradation under osmotic stress in continuously running sand columns. Other parameters of comparison were the expression of a catabolic gene involved in 4-chlorophenol degradation and the fatty acid composition of the membrane. It was found, that the dried cells were more tolerant towards harsh osmotic conditions and showed a better degradation performance than the fresh cells. Accordingly, their fatty acid membrane composition differed from that of the fresh cells, indicating a previous adaptation to drought during the formulation process. Along with high survival rates during this drying process, this study could further prove the applicability of *A. chlorophenolicus* A6 in bioaugmentation.

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PHYP026

Characterization of *Wolinella succinogenes* as a suitable host for the heterologous production of multihaem cytochromes *c*

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Multihaem cytochromes *c* represent one of the largest families of haem-containing proteins and play a central role in different metabolic pathways, especially in the biochemical nitrogen cycle. The availability of one or more covalently bound haem groups enable such proteins to function as electron carriers and redox enzymes. In addition, some multihaem cytochromes share structurally highly conserved haem group arrangements and exhibit associated enzymatic activities, although they catalyze different biochemical reactions.
We propose to combine heterologous expression of recombinant cytochromes *c* with structural and functional analyses. To illustrate this we have chosen to focus on multihaem cytochromes *c* such as pentahaem nitrite reductase (NrfA) and members of the hydroxylamine/hydrazine oxidoreductase family.
Wolinella succinogenes was chosen as host for the heterologous overproduction of these enzymes. This Gram-negative bacterium belongs to the Epsilonproteobacteria and has a high capacity to produce cytochromes *c* using a special system for bacterial cytochrome *c* maturation. The maturation system of *W. succinogenes* is called the Ccs system (cytochrome *c* synthesis) or system II and it occurs in β-, δ- and ε-proteobacteria, Gram-positive bacteria and cyanobacteria [1]. In contrast to the far more complex Ccm system (cytochrome *c* maturation) of α- and γ-proteobacteria the Ccs system involves only four proteins (CcsA, CcsB, CcdA, CcsX), which permit an efficient , heterologous overproduction of multihaem cytochromes *c* from other Epsilonproteobacteria such as pathogenic *Campylobacter* species [2]. Results of several multihaem cytochrome *c* production trials will be presented.

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Methods Enzymol. **486**, 429-446.

PHYP027

Deciphering the peroxide response of *Bacillus pumilus* by combining different "omics"-techniques

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The gram positive soil bacterium *Bacillus pumilus* is a possible candidate for the development of new production strains used in industrial fermentation processes. Bacteria living in soil are exposed to changing environmental conditions. Stress conditions may not only occur in natural habitats but also during fermentation processes where they would influence the growth of bacteria as well as product formation and product quality. To survive in their environment, bacteria have to adapt rapidly to unfavourable stress and starvation conditions. Adaptation to such conditions is controlled by complex regulatory networks and will be achieved by the induction of general and specific stress proteins and often down-regulation of proteins involved in basic metabolic processes.
The physiology of *B. pumilus* is quite similar to that of the closely related Gram-positive model organism *B. subtilis*. However, the reaction of both organisms to oxidative stress caused by hydrogen peroxide is different. Although *B. pumilus* is rather conspicuous because important proteins involved in detoxification of hydrogen peroxide, like KatA, are missing, the cells survive significantly higher amounts of this toxin.
We analyzed the response of *B. pumilus* to oxidative stress caused by hydrogen peroxide using transcriptomics, proteomics and metabolomics methods to resolve why the cells survive such high concentrations of this compound. The reprogramming of gene/ protein expression during stress was investigated as well as a possible influence of other low molecular weight thiol compounds.

PHYP028

A novel limonene dehydrogenase from *Castellaniella defragrans*

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Monoterpenes are ubiquitous occurring hydrocarbons mainly produced by plants. Despite its antimicrobial effects, the monocyclic unsaturated monoterpene limonene can be used as sole carbon source by some bacteria. Under oxic conditions, oxygen can be transferred to various positions of the ring or side chains, usually by a cytochrome P450 monooxygenase. The hydroxylation of the primary methyl group to perillyl alcohol is one of the most common activation processes of limonene. Under exclusion of oxygen, *Castellaniella defragrans*, a denitrifying betaproteobacterium, can also metabolize cyclic monoterpenes like limonene in addition to the myrcene pathway for acyclic monoterpenes. Similar to the aerobic activation of the cyclic limonene, the anaerobic oxidation also occurs at the primary methyl group. Via partial purification of the activity for the reaction of perillyl alcohol to limonene, two proteins related to phytoene desaturases were identified and the activity of the enzymes was verified through heterologous expression of the genes. Both adjacent genes are expressed in the presence of monoterpenes and are located in a genome island where most of the monoterpene related genes can be found. The proteins form heterodimers and enable *Castellaniella defragrans* to degrade monocyclic as well as bicyclic monoterpenes anaerobically. Results on the characterization of the purified proteins, including substrate specificity and kinetic properties, will be presented.

PHYP029

The W-/Se-containing class II benzoyl-coenzyme A reductase complex in *Geobacter metallireducens*

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In anaerobic bacteria most aromatic growth substrates are converted into the central intermediate benzoyl-coenzyme A (benzoyl-CoA). Benzoyl-CoA reductases (BCRs) dearomatize benzoyl-CoA to cyclohexa-1,5-diene-1-carboxyl-CoA (dienoyl-CoA). Obligately anaerobic bacteria such as *Geobacter metallireducens* employ the class II benzoyl-CoA reductase $[(BamBC)_2\ DEFGHI]_2$, which was recently purified and characterized[1]. This ~1 MDa complex contains tungsten, selenocysteines, FADs and several FeS-clusters. The active site components harboring the tungstopterin-cofactor, BamBC, were recently isolated and characterized[2]. Using homologously expressed BamBC with a Strep-tag the active site of the Benzoyl-CoA reducing subunit containing an unusually ligated tungsten was investigated in detail. Attempts to proof an electron bifurcation driven mechanism of ATP-independent class II benzoyl-CoA reductases were carried out.

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PHYP030

Organohalide respiration in *Sulfurospirillum multivorans*: Details of an unusual anaerobic respiratory chain revealed by genomics and proteomics

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The diversity of prokaryotic anaerobic respiration is enormous and a broad range of compounds is used as terminal electron acceptors in this process. One unusual form of anaerobic respiration is the utilisation of - often toxic or carcinogenic - halogenated compounds as electron acceptors, which are reductively dehalogenated in turn. Due to the harmful and persistent nature of many of these substrates, this type of respiration is also of a high environmental importance. One well-studied organohalide-respiring organism is the epsilonproteobacterium *Sulfurospirillum multivorans*. It uses the environmental pollutant tetrachloroethene (PCE) for organohalide respiration. PCE is persistent under oxic conditions, but is reductively dechlorinated to the more readily degradable dichloroethene (DCE) during organohalide respiration. The key enzyme of this process, the PCE reductive dehalogenase (PceA) is known to be a corrinoid-containing iron-sulfur protein, but the process of electron delivery to PCE as well as the molecular structure of the respiratory chain is still unknown.
We used comparative genomics together with differential proteomics to unravel the process of reductive PCE dehalogenation. In the first step, the genome of *S. multivorans* was sequenced, annotated and compared to other, non-dehalogenating *Sulfurospirillum* spp. genomes. Subsequently, proteomic studies were applied on cultures of *S. multivorans* grown with or without PCE. The presence of a ~50kbp region coding for enzymes taking part in PCE respiration was found solely in the genome of *S. multivorans* and synthesis of most of the according proteins was shown in cells grown with PCE only. Amongst other proteins, a putative quinol dehydrogenase was found in this region, which is thought to take part as an electron transfer

protein in the PCE respiratory chain. Our findings lead to a refined model of organohalide respiration in *S. multivorans*.
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PHYP031
Role of YqjA and YghB in the physiology of *Escherichia coli and Salmonella enterica LT2:* functional characterization of conserved membrane proteins

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Membrane integrity is the basis for all bacterial cells. Essential functions such as protein transport, signal transduction and energy generation all depend on an intact cytoplasmic membrane.
Importantly, two bacterial membrane proteins, called YqjA and YghB, have been described, which have a broad impact on cellular processes [1].
These proteins belong to a family of conserved polytopic membrane proteins, called the DedA protein family. Although the overall function of the DedA proteins is not known, initial genetic studies revealed that loss of YqjA and YghB resulted in effects on membrane phospholipid composition, protein transport, antibiotic resistance, cell division and adaptation to certain stress conditions [1]. Collectively, the DedA protein family seemed to be essential for the model bacterium *Escherichia coli*, which contains multiple copies of these conserved proteins.
Following these observations we believe that these proteins represent interesting new anti-bacterial drug targets. Currently we are investigating the function of YqjA and YghB in *E. coli* and *Salmonella enterica* LT2 and analyzing in more detail their impact on membrane protein assembly, protein transport and anaerobic respiration. Our latest results will be presented.

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PHYP032
Generating and characterization of an aldehyde ferredoxin oxidoreductase-negative *C. acetobutylicum* strain

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Clostridium acetobutylicum is a Gram-positive, endospore-forming, anaerobic soil bacterium, which is well known for its ability to naturally produce solvents, namely butanol and acetone. One aim of the "COSMIC2-project" (within the program Systems Biology of Microorganisms) is the construction of *C. acetobutylicum* mutants with artificially controlled genes for the production of solvents. Therefore, the "allelic exchange system" (ACE) system [1] will be used which is based on homologous recombination.
Previous transcriptome studies using microarray shift experiments showed an upregulation of several genes of solventogenic and acidogenic metabolism. One of those was the *aor* gene (CA_C2018) encoding an aldehyde:ferredoxin-oxidoreductase (AOR). AOR is an oxygen-sensitive enzyme that catalyzes the oxidation of aldehydes to their corresponding acids.
Using the ACE system, an *aor*-negative *C. acetobutylicum* strain was already constructed. Therefore, a knock-out cassette was generated by SOE PCR and then ligated into the pseudo-suicide vector pMTL-SC7515. The resulting plasmid was methylated in *Escherichia coli* pANS1, the methylated plasmid was transformed into *C. acetobutylicum*. After two independent homologous recombination events, a double-crossover *C. acetobutylicum* mutant was obtained. In further experiments, an (inducible) overexpression mutant of the *aor* gene will be created.
C. acetobutylicum wildtype and the *aor*-negative *C. acetobutylicum* mutant were characterized concerning growth and fermentation products. Compared to the *C. acetobutylicum* wildtype, the *aor*-negative *C. acetobutylicum* mutant was not able to grow on minimal medium and the production of acetone and butanol was dramatically reduced.

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PHYP033
Characterization of *Escherichia coli* mutant strains with a linear respiratory chain under different aerobiosis conditions

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E. coli is a very versatile microorganism that is able to scope with a variety of growth conditions. In this study the ability to grow with and without oxygen, as well as under low oxygen (microaerobic) conditions was investigated in a systematic manner in steady-state chemostat experiments. The respiratory chain of *E. coli* is branched to allow the cells flexibility to deal with changing environmental conditions. It consists of the main NADH:ubiquinone oxidoreductases NADH dehydrogenase I and II, as well as of three terminal oxidases, cytochrome oxidase bo, bd-I and bd-II. They differ with respect to energetic efficiency (proton translocation) and the affinity to the quinones and the end-acceptor.
A set of isogenic mutant strains was created, which lack NADH dehydrogenase I as well as two of the terminal oxidases, resulting in strains with a linear respiratory chain. These strains were analyzed in glucose-limited chemostat experiments under steady-state conditions with a defined oxygen supply, adjusting aerobic, anaerobic and microaerobic conditions.
The acetate formation rate decreased in the wild-type MG1655 linearly with increasing aerobiosis, without any by-product excretion at 100 % aerobiosis (as defined before). The mutant strains in contrast produced acetate even under aerobic conditions (like strain TBE029, which lack NADH dehydrogenase I but still contained the cytochrome oxidases). Strain TBE032, without NADH dehydrogenase I and with cytochrome oxidase bd-II as sole terminal oxidase showed the highest acetate formation rate under aerobic conditions. This supports its role as main oxidase under very limiting oxygen conditions, not able to catalyze the efficient reduction of the quinone pool at higher oxygen condition. The composition of the different quinone species varies over the aerobiosis, with ubiquinone as the main quinone under aerobic conditions. The ratio of the quinone species differed in some of the mutant strains. The phosphorylation of ArcA, the regulator of the two-component system ArcAB, beside Fnr one of the global transcription factors for the response towards different oxygen concentrations, was studied and showed a changed phosphorylation pattern in the mutant strains.

PHYP034
Heme d_1 biosynthesis and insertion into cytochrome cd_1 nitrite reductase in *Pseudomonas aeruginosa*

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The isobacteriochlorin heme d_1 is a unique tetrapyrrole cofactor occurring in the periplasmic cytochrome cd_1 nitrite reductase (NirS) of denitrifying bacteria such as *Pseudomonas aeruginosa*. While the covalently bound heme *c* is incorporated to NirS by the cytochrome *c* maturation machinery, less is known about the insertion of the non-covalently bound heme d_1 into the active site of the nitrite reductase. In the course of heme d_1 biosynthesis a precursor thereof needs to be transported through the inner membrane of the bacterial cell into the periplasm where the cofactor is finally inserted into NirS. The periplasmic proteins NirF and NirN, that are both able to bind heme d_1, are thought to be involved in the last step of heme d_1 biosynthesis and heme d_1 insertion into NirS, respectively.[1,2] A deletion of *nirF* in *P. aeruginosa* leads to an inactive nitrite reductase lacking heme d_1, whereas the deletion of *nirN* only results in a mild growth phenotype[3].
UV-Vis absorption spectra of the periplasmic fraction of a *P. aeruginosa* Δ*nirN* mutant suggested that heme d_1 insertion into NirS is strongly dependent on the presence of NirN. Additionally we showed that, in contrast to most other denitrifying bacteria[1], the heme d_1 binding protein NirF from *P. aeruginosa* is a lipoprotein attached to the inner membrane. Co-immunoprecipitation experiments revealed a protein-interaction network consisting of NirN, NirF and NirS. Furthermore, we identified additional protein interaction partners of the NirF-NirN-NirS complex through immunoaffinity chromatography after *in vivo* protein cross-linking and subsequent MS analysis of the eluted proteins. Several potential transport proteins possibly involved in the transport of a heme d_1 precursor across the membrane were detected.

Based on our results we propose a model for the final step of heme d_1 biosynthesis and the insertion of heme d_1 into NirS. In this model the proteins NirF, NirN and NirS form a membrane attached complex. NirF takes up the heme d_1 precursor from the transporter and catalyzes the last step of heme d_1 biosynthesis. The cofactor is then inserted into NirS with NirN playing a so far uncharacterized assisting role for the insertion process.

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PHYP036
Posttranslational modification of indolelactyl-CoA dehydratase in *Clostridium sporogenes*

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Clostridium sporogenes, an anaerobic intestinal bacterium, degrades tryptophan via the reductive branch of a Stickland reaction to 3-indolepropionic acid (IPA), which as an excellent oxygen radical scavenger protects the human brain from Alzheimer's disease. It has been proposed that after transamination of tryptophan the resulting 3-indolepyruvate is reduced to (*R*)-3-indolelactate and further dehydrated to (*E*)-indoleacrylate, which finally is reduced to 3-indolepropionate. In this work all intermediates and the activities of the enzymes from this proposed pathway were determined, as are tryptophan aminotransferase, 3-indolelactate dehydrogenase, a complex composed of 3-indolelactate CoA-transferase (FldA) and 3-indolelactyl-CoA dehydratase (FldBC), the activator of the dehydratase (FldI), and indolacrylate reductase. Indolelactyl-CoA dehydratase, the key enzyme of this pathway, catalyzes the reversible *syn*-elimination of H_2O with an unusual one-electron transfer radical mechanism [1]. The enzyme also catalyzes the dehydration of 3-phenyllactyl-CoA derived from phenylalanine [2] and of 4-hydroxy-3-phenyllactyl-CoA from tyrosine. Though encoded by the same genes, the dehydratase purified from cells grown on tryptophan exhibited a higher catalytic efficiency (k_{cat}/K_m) with 3-indolelactyl-CoA than with 3-phenyllactyl-CoA, whereas k_{cat}/K_m of the dehydratase from cells grown on phenylalanine was higher with 3-phenyllactyl-CoA than with 3-indolelactyl-CoA. Surprisingly the molecular masses of both subunits of the dehydratases are smaller than predicted from their genes (*fldB* and *fldC*). By expression of *fldBC* in *Escherichia coli*, however, an even more active dehydratase was obtained of which both subunits possessed their predicted masses (FldB, 46.4 kDa, FldC, 43.2 kDa). N-Terminal sequencing suggested that in *C. sporogenes* a specific protease cuts peptides from the C-termini of FldB and FldC. Further experiments have to resolve, whether the extent of this apparent posttranslational modification depends on the amino acid used for growth.

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PHYP037
Identification and characterization of tetrachloroethene respiratory chain components in *Sulfurospirillum multivorans*

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Sulfurospirillum multivorans, a gram-negative epsilonproteobacterium, performs a special type of anaerobic respiration. The organism is able to couple the reductive dechlorination of tetrachloroethene (PCE) to energy conservation via electron transport phosphorylation (organohalide respiration). Very little is known about the composition and topology of the PCE respiratory chain. The terminal oxidoreductase is the PCE reductive dehalogenase (PceA), which harbours a corrinoid and two iron-sulphur clusters as cofactors. The superreduced state of the corrinoid cofactor is required for PCE reductive dechlorination. With H_2 or formate as electron donor, cytochrome *b* and a quinone should be components of the respiratory chain. The inhibition of PCE respiration by 2-heptyl-4-hydroxy quinoline-N-oxide (HQNO) supports the assumption that a quinone is involved. The electron transport from quinones to the corrinoid is enigmatic and was in the focus of our studies.
For these studies differential proteome analysis was conducted. This technique allowed for the identification of a PCE-induced quinol dehydrogenase among a set of other proteins specifically produced in PCE-grown cells. To identify the quinones involved in electron transfer of cells grown with different electron acceptors, quinone extraction and analysis were carried out (cooperation with S. Baumann, UFZ Leipzig). The PCE reduction was tested in *S. multivorans* cells in the absence or presence of reduced quinone analogues (e. g. Menaquinone-4). These experiments were extended by the investigation of the electron transfer pathway within the PceA enzyme. Therefore PceA was purified and subjected to electron paramagnetic resonance spectroscopy (EPR) to determine the midpoint redox potentials of the metallo-cofactors. The results of this study lead to an advanced model of the PCE respiratory chain in *S. multivorans*.

PHYP038
Sulfite oxidation in the purple sulfur bacterium *Allochromatium vinosum*

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Although sulfite is an intermediate during the oxidation of reduced sulfur compounds to sulfate in the cytoplasm of all phototrophic sulfur bacteria and several purple sulfur bacteria can even use externally available sulfite as photosynthetic electron donor, the exact mode of sulfite oxidation in these organisms is a long-standing enigma. Indirect oxidation via adenosine-5'-phosphosulfate (APS) catalyzed by APS reductase and ATP sulfurylase is a well described pathway, however, it is neither generally present nor is it essential [1]. Strong inhibition of sulfite oxidation by tungstate in the purple sulfur bacterium *Allochromatium vinosum* [1] indicated the involvement of a molybdenum-containing enzyme in the process but none of the genome-sequenced purple or green sulfur bacteria have homologs of genes for the characterized periplasmic sulfite-oxidizing molybdoproteins SorAB or SorT. However, all sequenced phototrophic sulfur bacteria contain genes for a polysulfide reductase-like protein consisting of a membrane-anchor and one or two cytoplasmic subunits. The latter are an iron-sulfur protein and a molybdoprotein or a fusion thereof. Here, we show that an *A. vinosum* strain carrying an insertional mutation in the gene for the active-site molybdopterin-containing subunit excretes massive amounts of sulfite en route to sulfate when grown on sulfide and exhibits 83±10% reduction of the oxidation rate as compared to the wild type when the cells are grown on sulfite. A double mutant that lacks APS reductase in addition exhibits an even more dramatic impairment, albeit the strain still has the capacity to completely oxidize all sulfur present initially to sulfate. This ability was finally lost in a triple mutant in which the *soxY* gene was deleted in addition. This gene encodes a subunit of the periplasmic substrate-binding protein SoxYZ, which is part of the thiosulfate-oxidizing Sox enzyme system. A mutant strain carrying only the *soxY* deletion also exhibited a strong phenotype with regard to sulfite oxidation irrespective of the presence of two functional cytoplasmic sulfite-oxidizing systems. While the exact function of SoxYZ in sulfite oxidation remains elusive at this point, at least part of its importance could be due to a role as a periplasmic sulfite-binding protein.

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PHYP039
Reductive defluorination by ATP-dependent benzoyl-CoA reductases

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Halogenated aromatic compounds are widely distributed in the environment and can be fully degraded by microorganisms under aerobic and anaerobic conditions. *Thauera chlorobenzoica* 3CB-1 is able to grow with 3-chloro- or 3-bromobenzoate as the sole source of carbon and energy under denitrifying conditions. After activation to the corresponding CoA-thioesters they are reductively dehalogenated to benzoyl-CoA and HCl/HBr by ATP-dependent benzoyl-CoA reductases[1]. Benzoyl-CoA is then further metabolized to acetyl-CoA and CO_2 by enzymes of the benzoyl-CoA degradation pathway. Due to mechanistic reasons 3-fluorobenzoyl-CoA was rather dearomatized to a fluorinated 1,5-dienoyl-CoA dead end product, and consequently *T. chlorobenzoica* cannot use 3-F-benzoate as growth substrate. In this study

we demonstrate that *Thauera aromatica* K172 can grow with 4-F-benzoate as the only source of carbon and energy. In extracts grown on 4-F-benzoate, the growth substrate was first activated to the corresponding CoA-thioester, followed by an electron donor- and ATP-dependent defluorination. The defluorinating enzyme was highly enriched from extracts of cells grown on 4F-benzoate and identified as an ATP-dependent benzoyl-CoA reductase. The ratio of benzoyl-CoA dearomatization/4-fluorobenzoyl-CoA defluorination activities was constant in the course of the enrichment, indicating that the both activities were catalyzed by the same enzyme. The results obtained indicate that ATP-dependent class I benzoyl-CoA reductases have an intrinsic reductive dehalogenation activity with a high specificity for individual halogen atoms at individual positions at the aromatic ring.

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PHYP040

Electron Transferring Flavoprotein: Menaquinone Oxidoreductase in *Geobacter metallireducens*

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Electron transferring flavoproteins (ETF) function as electron acceptor for various enoyl-CoA forming acyl-CoA dehydrogenases. Electrons are then transferred to a respiratory quinone. In organisms using ubiquinone the enzyme ETF: ubiquinone oxidoreductase mediates the electron transfer between ETF and quinone and was studied intensively (1). However, this enzyme is absent in the genome of many procaryotes using menaquinone instead of ubiquinone like the obligately anaerobic deltaproteobacterium *Geobacter metallireducens*. This organism uses many aromatic compounds as growth substrates and channels most of them to the central intermediate benzoyl-CoA. During benzoyl-CoA degradation glutaryl-CoA dehydrogenase converts glutaryl-CoA to crotonyl-CoA and electrons were supposed to be transferred via ETF and a yet unknown ETF: menaquinone oxidoreductase to menaquinone (2). In this study we expressed ETF heterologously and demonstrated its interaction with glutaryl-CoA dehydrogenase. The recently established genetic system for *G. metallireducens* (3) was used to homologously express a gene predicted to code for an ETF: menaquinone oxidoreductase. Solubilization and purification of the Strep-tagged membrane protein were achieved and some of its properties will be presented.

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PHYP041

Interaction studies between HemC and HemD of *Bacillus megaterium* involved in tetrapyrrole biosynthesis

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In all organisms the biosynthesis of tetrapyrroles involves several enzymatic steps. After the formation of 5-aminolevulinic acid (ALA) and porphobilinogen (PBG), the porphobilinogen deaminase HemC acts as the third enzyme in this pathway. It oligomerizes four PBG molecules to the linear intermediate pre-uroporphyrinogen. As this intermediate is prone to autocyclization to the toxic uroporphyrin I upon solvent exposure, a complex formation between HemC and the next enzyme, the uroporphyrinogen III synthase HemD, is suggested [1], but could never be shown. This assumption is further strengthened by the fact that in many organisms the genes *hemC* and *hemD* are located in one operon implying an equimolar and simultaneous expression.
The proteins HemC and HemD of *Bacillus megaterium* were overproduced in *Escherichia coli* and could be purified via affinity chromatography in high amounts. Monoclonal antibodies against both proteins were obtained and their specificity tested. For determination of the complex formation, His-HemC and HemD could be co-purified via affinity chromatography confirming experimentally the *in vitro* interaction for the first time. Further, gel permeation chromatography (GPC) studies were performed to prove the *in vitro* complex formation of HemC and HemD. As a *in vivo* method a Bacterial Two Hybrid Assay in *E. coli* with the *Bacillus megaterium* proteins HemC and HemD was used also giving evidence for interaction. Additionally, HemC and HemD were fused to the yellow (YFP) and cyan fluorescent protein (CFP), respectively, in order to show *in vivo* the interaction via fluorescence resonance energy transfer (FRET) in the original host *B. megaterium*. Bioinformatic modelling and first attempts to crystallize the protein complex were made to investigate the interaction in more detail.

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PHYP042

Physiological role of nitric oxide (NO) in the denitrifying methanotroph *Candi-datus* Methylomirabilis oxyfera

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Nitric oxide (NO) is a central intermediate in the nitrogen metabolism of denitrification and anammox, and furthermore plays an important role in intracellular signalling. Due to its high toxicity, mechanisms have evolved to ensure its detoxification to nitrous oxide (N_2O), so that the turnover is rapid and significant molecular responses occur at very low concentrations.
The anaerobic methanotroph *Methylomirabilis oxyfera* employs a novel pathway of nitrite conversion in which, after the canonical reduction of nitrite to nitric oxide, the latter is most likely being dismutated to molecular nitrogen and oxygen (Ettwig et al., 2010). This reaction has never been observed in nature and is not yet understood at the molecular level. The produced oxygen is then used to activate methane in a monooxygenase reaction.
M. oxyfera encodes and expresses a cytochrome *cd1* nitrite reductase (NirS) and five gene paralogs of qNor-like nitric oxide reductases. Nitrous oxide reductase encoding genes are absent from the genome. The two most highly expressed paralogs, Nod1 and Nod2, lack essential features necessary for nitrous oxide formation, and have been proposed to be responsible for the dismutation of nitric oxide (Ettwig et al., 2012). So far it is unknown whether nitrous oxide may be formed as a result of an unbalanced NO supply.
The physiological response of an *M. oxyfera* enrichment culture was monitored after controlled additions of nitric oxide in a continuous membrane reactor set-up. The concentrations of nitrogenous compounds were determined throughout the experiment and biomass samples were used for RNA extraction. The global gene expression will be analysed after mRNA sequencing by Ion Torrent technology. The potential of nitric oxide in metabolic regulation of the intra-aerobic denitrification pathway of *M. oxyfera* will be discussed.

Ettwig *et al*. (2010): Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. *Nature* **464**: 543-548
Ettwig *et al*. (2012): Bacterial oxygen production in the dark. *Front. Microbio*. **3 Article 273**: 1-8

PHYP043

Two pathways of autotrophic CO_2 fixation in *Ammonifex degensii*

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All life on earth depends on the fixation of inorganic, atmospheric carbon dioxide into an organic form that can be utilized in biosynthesis. Currently, six different pathways for carbon fixation operating in plants, algae, bacteria and archaea have been described. Analysis of prokaryotic genomes suggests that some bacterial and archaeal species employ more than one pathway for carbon fixation [1]. However, experimental evidence for the co-existence of two autotrophic pathways in one organism has only been obtained for an uncultured endosymbiont of a deep-sea tube worm. This symbiont apparently synthesizes the key enzymes for either the Calvin-Benson cycle or the reductive citric acid cycle depending on the energy situation [2].
Ammonifex degensii, a member of the low GC, gram-positive bacteria, possesses genes coding for key enzymes of the reductive acetyl-CoA pathway as well as for an archaeal form III ribulose-1,5-bisphosphate oxygenase/carboxylase (Rubisco) and a phosphoribulokinase. Interestingly,

the genes for Rubisco und phosphoribulokinase as well as six other genes encoding enzymes of the Calvin-Benson cycle are organized in an operon, which was confirmed using RT-PCR. Three enzymes from this operon, Rubisco, phosphoribulokinase and fructose-1,6-bisphosphate aldolase/phosphatase were expressed heterologously in *Escherichia coli* and showed catalytic activity *in vitro*. This suggests that they are part of a functional Calvin-Benson cycle. Measurements of *A. degensii* cell extract showed the activities of key enzymes of both the Calvin-Benson cycle and the reductive acetyl-CoA pathway. While details regarding the regulation of carbon metabolism still need to be evaluated, these findings are the first evidence for the coexistence of both the reductive acetyl-CoA cycle and the Calvin-Benson cycle in a single organism as well as for the involvement of archaeal form III ribulose-1,5-bisphosphate oxygenase/carboxylase in carbon fixation.

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[2] S. Markert *et al.*, *Science* 315, 247-250 (2007)

PHYP044

First biochemical characterization of a novel-type oxygen-tolerant [NiFe]-hydrogenase

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Within the global hydrogen cycle, soil deposition is the most important natural process for the removal of H_2 from the atmosphere. However, the mechanism of H_2-uptake remained elusive. Recently, a novel class of [NiFe]-hydrogenases was found mainly in members of the phylum Actinobacteria [1]. Several species possessing this hydrogenase were shown to be able to oxidize H_2 at atmospheric levels (approx. 0.5 ppmv). It was proposed that they are responsible for hydrogen uptake in soils [2].
Interestingly, a hydrogenase of this class is also encoded in the genome of the β-proteobacterium *Ralstonia eutropha* H16 [3]; it was designated "Actinobacterial Hydrogenase" (AH). This hydrogenase is encoded in a conserved operon structure, which contains structural genes for a small and a large subunit, a complete set of hydrogenase maturation genes as well as 4-5 conserved unknown genes. Here we present a biochemical characterization of the AH from *R. eutropha*, displaying the first detailed investigation on a [NiFe]-hydrogenase of the novel "group 5"-type.
The AH is active under standard conditions as shown by in-vivo H_2-uptake measurements and via activity staining of soluble extracts separated by native PAGE. To facilitate purification, an overexpression strain was constructed in which the AH was modified with a *Strep*-tag II affinity peptide. Pure AH_{Strep} protein was obtained by *Strep*-Tactin affinity chromatography, showing bands of two subunits with 65kDa and 37kDa when separated on SDS-PAGE. Gel permeation chromatography revealed a size of 144±6 kDa for the the monomeric form of the AH; a dimeric form was also found. The pH- and temperature optimum of H_2-uptake were determined photometrically with nitroblue tetrazolium chloride (NBT) as electron acceptor. Amperometric measurements in a modified Clark-electrode showed that the AH is insensitive to oxygen. A relatively high K_m of 3.6 ± 0.5 μM H_2 was determined for H_2-uptake, what puts doubt on the assumption that the AH from *R. eutropha* participates in H_2-uptake in soils. Further investigations are under way to uncover the physiological role and mechanistic properties of the AH.

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PHYP045

The function of SoxL in the purple sulfur bacterial thiosulfate-oxidizing Sox system

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In the purple sulfur bacterium *Allochromatium vinosum* thiosulfate oxidation to sulfate occurs via intermediary formation of intracellular sulfur globules and is strictly dependent on the heme *c*-containing SoxXAK, the thiohydrolase SoxB and the substrate-binding protein SoxYZ. After SoxXAK-catalyzed binding of thiosulfate to cysteine 152 of SoxY and subsequent release of sulfate by SoxB, SoxYZ is thought to be regenerated for the next reaction cycle by a sulfurtransferase-catalyzed transfer of the remaining SoxY-bound sulfane sulfur to growing sulfur globules.
One obvious candidate for this function is the rhodanese-like protein SoxL. This protein is neither essential for sulfur globule formation from thiosulfate in *A. vinosum* strain DSM 185 [1] nor in strain DSM 180 (this work) suggesting that other periplasmic sulfur transferases (e.g. Alvin_0258) act as back-up systems. However, a relevant function of SoxL in the Sox cycle is supported by two independent lines of evidence: (1) The *soxL* gene is localized immediately downstream of *soxXAK* and here we prove co-transcription of *soxK* and *soxL*. (2) The *in vitro* electron transfer rate from thiosulfate to cytochrome *c* in the presence of *A. vinosum* SoxXAK, SoxB and SoxYZ is significantly enhanced in the presence of SoxL [2]. Here, we show that both conserved potential active site cysteine residues of SoxL are essential for this function.
Experiments with the fluoresecent thiol-reactive reagent 1,5-I-AEDANS showed that only one of these residues, Cys_{184}, is surface exposed while the second (Cys_{179}) appears to be structurally hidden. After exposure of SoxL to sulfide, Cys_{184} is no longer reactive with 1,5-I-AEDANS indicating that either persulfurated Cys_{184} is no longer surface-exposed or occluded by formation of a bridge of one or more sulfur atoms with a second cysteine, possibly Cys_{179}. When incubated with sulfur-loaded SoxYZ, SoxL behaved exactly as observed with sulfide alone while a mutated SoxYZ protein lacking the substrate-binding cysteine ($Cys_{152}S$) did not cause any effect on SoxL. Taken together our findings provide strong evidence for a specific interaction of and probable sulfur transfer between SoxYZ and SoxL.

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[2] Welte et al. (2009) FEBS Lett. 583, 1281-1286.

PHYP046

Antifungal susceptibility testing based on the bioluminescence by *Armillaria cepistipes*, formely unknown to produce light

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The phenomenon of bioluminescence of fireflies, bacteria, and dinoflagellates is known for a long time. Already in ancient times philosophers and scientists studied the light production and emission by living organisms. However, very little research has been carried out on fungal bioluminescence. Today, more than 70 higher fungal species are known to be bioluminescent and new light emitting species are discovered continuously. Luminescent fungi can have a great practical value using them as eukaryotic biosensors for the detection of pollutants and antifungal agents. At this time, all known bioluminescent fungi are Basidiomycetes belonging to four distinct lineages. However, data are available only for a few well known species, including *Armillaria mellea, Mycena citricolor, Mycena chlorophos*, *Omphalotus olearius* and *Panellus stipticus*. Luminescence may be present in various parts of the fungi. In many bioluminescent *Mycena* and *Armillaria* species, only the mycelium emits light. In others both the mycelium and the basidioms are luminescent, as for example in *P. stipticus* and *O. olearius*. To study the effect of several antifungal agents, we developed a biotest based on the light production of *A. cepistipes*, which was - until today - not known be a light-emitting fungal species. As example, mycelium was exposed to beta-Thujaplicin at concentrations of 0.1, 1, 10, and 100 mg/l and the subsequent alteration of the light emission was measured. We compared the results also regarding the influence of the toxin on fungal biomass production. At high concentrations, beta-Thujaplicin negatively affected luminescence as well as biomass formation. Surprisingly, at low toxin concentrations a stimulation of light emission and biomass production was detectable suggesting hormetic effects of beta-Thujaplicin. In summary, this bioassay provides a rapid and easy practicable method to test possible antifungal substances. It might be particularly useful to find new agents that inhibit the *in vivo* growth of *Armillaria*, because several *Armillaria* species are known to be severe forest pathogens.

PHYP047

Diversity of corrinoids extracted from reductively dehalogenating *Desulfitobacterium hafniense* strains Y51 and DCB-2

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Corrinoids are known to accomplish reductive dehalogenation either abiotically as pure chemical or biotically as cofactor of reductive dehalogenase enzymes (Rdhs). Almost 10 years ago the structure of the corrinoid cofactor of the tetrachloroethene (PCE) reductive dehalogenase found in *Sulfurospirillum multivorans* (epsilonproteobacterium) was shown to be a unique Norpseudo-B_{12} [1]. The organism displayed the ability to synthesize corrinoids *de novo*, like other, but not all, anaerobic reductively dehalogenating bacteria. The structural variation between Norpseudo-B_{12} and the frequently found Vitamin B_{12} launched a discussion on the diversity of corrinoid cofactors present in Rdhs.
Natural or man-made organohalides are reductively dehalogenated by several species among the Desulfitobacteria (phylum Firmicutes). The organisms are able to convert either aliphatic or aromatic halogenated organic compounds. Only a few corrinoid-dependent Rdhs were purified from the organisms and characterized biochemically. So far no information is available about the corrinoid cofactors of these enzymes. The ability for *de novo* corrinoid biosynthesis is found in many *Desulfitobacterium* strains. Besides the corrinoid biosynthesis genes, the genome sequences of strain Y51 and DCB-2 harbored different genes encoding reductive dehalogenases with varying substrate specificity.
In this study *D. hafniense* Y51 and DCB-2 were cultivated with different organohalides (tetrachloroethene and 3-chloro-4-hydroxyphenylacetate or 2,4,6-trichlorophenol, respectively) and subjected to corrinoid extraction. For both organisms different corrinoids have been detected that were analyzed further using LC-MS. In addition, the impact of exogenous Vitamin B_{12} on the organohalide-dependent growth and reductive dehalogenase activity was tested. The modulation in corrinoid diversity by supplementing benzimidazole analogues (e. g. 5,6-dimethylbenzimidazole), that might be incorporated as lower ligand bases into the corrinoid structure, was investigated as well. A first insight into the variability in corrinoid cofactors produced within the *Desulfitobacterium* genus will be presented that might lead to a better understanding on the requirements for reductive dehalogenation in nature.

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PHYP048

Computational Models of Cyanobacterial Metabolism: Systemic properties of phototrophic growth

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Cyanobacteria are the only known prokaryotes with the capability to perform oxygenic photosynthesis and contribute significantly to the global oxygen, nitrogen and carbon cycles. Due to their highly versatile metabolism and their ability to directly convert solar energy into hydrocarbons, biotechnological applications of cyanobacteria are at the forefront of several current global challenges, such as the supply of energy from non-fossil resources and the efficient sequestration of atmospheric CO_2. The domestication of phototrophic microorganisms remains one of the grand challenges of the 21st century.
One step towards such a domestication of cyanobacteria is an integrated experimental and computational approach to understand the functional properties of phototrophic growth.The focus of the contribution is to describe the construction of computational models of cyanobacteria, from single pathways to an entire organism, and the analysis of such models using kinetic and contraint-based methods. Specifically, we present an extended reconstruction of the metabolic network of the unicellular cyanobacterium *Synechocystis* sp. PCC 6803. Building upon several recent reconstructions of cyanobacterial metabolism, unclear reaction steps are experimentally validated and the functional consequences of unknown or dissenting pathway topologies are discussed.
The updated model is analysed computationally with respect to the metabolic re-organization during diurnal cycles of light availability.

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PHYP049

The role of desulfitobacteria in degradation of lignin-borne methoxylated chloroaromatics in forest soil

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Lignin-degrading fungi of boreal forests show the ability to produce halogenated organic compounds while growing on wood. Such natural organohalides were proposed to be growth substrates for dehalogenating soil bacteria. Members of the genus *Desulfitobacterium* (Firmicutes) were described to reductively dehalogenate a variety of chlorinated or brominated organic compounds under anoxic conditions [1]. The *Desulfitobacterium* spp. are ubiquitous in terrestrial ecosystems and were frequently isolated from soil contaminated with man-made organohalides [2]. However, the function of these anaerobic bacteria in the microbial food web responsible for the degradation of lignin-borne methoxylated chloroaromatics is poorly characterized. To unravel their role pyrosequencing approaches were used in this study to elucidate the composition of the microbial community at hot spots of lignin degradation. In parallel enrichments from beech wood forest soil fed with the fungal metabolite 3,5-dichloro-4-methoxybenzoate were carried out. Bacterial growth was monitored using a newly designed qPCR assay. Within one transfer of the cultures an enrichment of *Desulfitobacterium* spp. from 0.01% to 0.1% of the total bacterial community was observed. Metabolite analysis via LC-MS revealed that *O*-demethylation of the substrate 3,5-dichloro-4-methoxybenzoate precedes its dechlorination. *Desulfitobacterium* spp. are equipped with different *O*-demethylase genes as well as several genes encoding reductive dehalogenases. The results gained in this study support the hypothesis that *Desulfitobacterium* spp. couple *O*-demethylation to reductive dechlorination in a way that the methoxy-group is used as electron donor.

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PHYP050

Degradation of benzo[a]pyren by bacterial isolates from human skin

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As a host-microbe interface the human skin is not only a breeding ground to a most diverse and dense population of microbial commensals but at the same time it is the organ most exposed to polycyclic aromatic hydrocarbons such as benzo[a]pyrene (BaP). In eukaryotes cytochrome P450-mediated activation of the latter is a model for metabolism-mediated carcinogenesis. Meanwhile, the oxidative degradation of BaP by microbes is less well studied. The respective intermediates are often unknown let alone characterised toxicologically. This study now reports the isolation of several BaP-degrading microbes from several different habitats on human skin. Degradation of BaP proceeded via oxygenative ring cleavage and was complete in 4 out of 10 isolates. Substrate metabolism involved several transcripts, two of them encoding for enzymes with sequence similarities to biphenyldioxygenase (*bphA*). Analysis of the 16s-DNA sequences showed that whilst the isolated BaP-degrading cultures comprised Gram+ as well as Gram- bacteria Micrococci were predominant.

PHYP051

Electron transferring flavoprotein from *Megasphaera elsdenii*: Unraveling a fifty years old enigma.

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The Gram-negative anaerobic bacterium*Megasphaeraelsdenii,* order *Clostridiales,*wasreported to ferment lactate to CO_2, H_2, acetate, propionate, butyrate and valerate. A key position in this pathway holdsthe 2 FAD-containing electron transferring flavoprotein (EtfAB) that transports electrons from the FAD-containing lactate dehydrogenase or from NADH to the enoyl-CoA reductase related to acyl-CoA dehydrogenases. In *Clostridium kluyveri, Clostridium tetanomorphum* and *Acidaminococcusfermentans* it has been shown that EtfAB together with butyryl-CoA dehydrogenase (Bcd) bifurcates electrons from 2 NADH to crotonyl-CoA and ferredoxin yielding butyryl-CoA and the 'energy rich' reduced ferredoxin [1,2,3]. This coupling between the reductions of crotonyl-CoA and ferredoxin is very tight; in the absence of ferredoxin no butyryl-CoA is formed.
Till the end of the decade it was made to believe in over a half a dozen publications that purified ETF from *M. elsdenii* in combination with butyryl-CoA dehydrogenase appears to mediate the reduction of crotonyl-CoA by NADH without ferredoxin [4]. To check this discrepancy, we overproduced EtfAB and Bcd from *M. elsdenii* in *Escherichia coli* and performed with the affinity purified proteins the bifurcation assay, in which hydrogenase was included to regenerate oxidized ferredoxin, according to the equation: 2 NADH + 2 H^++ crotonyl-CoA ® 2 NAD^+ + H_2 + butyryl-CoA. Under anoxic conditions this assay worked well; in the presence of air, however, the rate of NADH oxidation was much faster and independent of whether crotonyl-CoA or butyryl-CoA was added. We conclude that NADH mediated by Etf reduces Bcd, which upon addition of crotonyl-CoA or butyryl-CoA suffers a conformational change that opens an access for oxygen to $FADH_2$ generating superoxide anion $O_2^{\bullet-}$ and/or H_2O_2. Hence the reported function of Etf in *M. elsdenii* measured under air [4] turned out to be an artifact.

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PHYP052

Microbial degradation of cyclic peptides: Novel substrates, analytics, isolates and insights

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Diketopiperazines (DKPs) are cyclic dipeptides and represent a widespread class of natural compounds produced by a variety of organisms. They are described to act as antibacterial, antifungal, antiviral or cytostatic agents but also represent an additional class of bacterial quorum sensing messengers [1].
In this study the microbial degradation and enzymatic cleavage of these molecules was investigated. Eleven DKPs were examined, eight of which were composed of proteinogenic amino acids and three of non-proteinogenic amino acids.
An HPLC analysis was established for the quantification of DKPs and their potential degradation products (linear dipeptides) detecting and separating the underivatized substances [2]. These HPLC methods have been applied to test and identify potential biocatalysts for DKP degradation:
- Peptidase catalyzed hydrolysis of certain DKPs formerly reported in literature was disproved.
- Five bacterial strains were newly identified for DKP degradation. Two of these strains had been isolated during this study and were classified as *Microbacterium* sp. and *Paenibacillus* sp. by 16S rDNA sequence analysis and alignment.
- Investigating these novel isolates, enantioselective hydrolysis of a racemic DKP [cyclo(DL-Ala-DL-Ala)] was demonstrated for the first time.
- The first microbial degradation of the DKPs cyclo(L-Ala-L-Ala), cyclo(D-Ala-L-Ala) and cyclo(L-Asp-L-Asp) was demonstrated.

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PHYP053

Engineering *Methanosarcina* for conversion of carbohydrates to methane

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Anaerobic biomass degradation to methane and carbon dioxide involves syntrophic interactions between (micro)organisms from all three domains of life. The last step in this process is catalyzed exclusively by methanogenic archaea. Though energetically feasible no methanogen is known capable of converting exogenous carbohydrates to methane. As *Methanosarcina acetivorans* appears to encode all functions necessary for glycolytic methanogenesis, lacking only functions for glucose uptake and glucose activation, we amended *M. acetivorans* with these functions. However, utilization of methanol as growth substrate in the presence of glucose was impaired in the respective transgenic strains, which indicates that the synthetic glycolytic trait interferes with energy metabolism. Consequently, the trait was rapidly lost as no positive selection for it could be applied. To overcome this selective pressure against the synthetic pathway, it was established in a *M. acetivorans* mutant lacking carbon monoxide dehydrogenase/acetyl-CoA synthase which is required for autotrophy. The resulting strain is auxotrophic for acetate but also was able to utilize glucose as carbon source. This strain will now allow for optimization of the synthetic glycolytic pathway by evolutionary engineering.

PHYP054

Beta beware: Microbial degradation of aromatic beta-amino acids

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Chiral beta-amino acids are valuable building blocks for the production of several fine chemicals and important pharmaceuticals e.g. taxanes (cytostatic drugs against breast cancer, annual sale >2 bill. €). As their chemical synthesis is still inefficient, our approach is to investigate the bacterial metabolization of these compounds as nature usually provides enzymes for the synthesis of most compounds which can be biologically degraded.
Using racemic beta-phenylalanine as model compound, the initial conversion turned out to be an *(S)*-selective reaction to the corresponding beta-keto acid by inducible PLP-dependent transaminases in all bacteria tested so far [1]. We established a chiral HPLC analysis suitable for the enantioseparation of several aromatic beta-amino acids to study the substrate spectrum of these enzymes [2].
We demonstrated that beta-phenylalanine, beta-tyrosine and para-chloro-beta-phenylalanine but not homophenylalanine can serve as sole nitrogen source supporting the growth of a model organism. We are now trying to purify the responsible biocatalyst(s) to find out whether the observed transamination activity relies on only one enzyme. Such a promiscuous "beta-transaminase" might serve as an important tool for the chemo-enzymatic production of aromatic beta-amino acids [3].

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PHYP055

Different ecophysiologies of two *Dehalococcoides mccartyi* strains enriched from the Bitterfeld Region of Central Germany

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Soils, groundwater and sediments of the Bitterfeld area in central Germany are highly contaminated by different chlorinated compounds as a result of discharges from the chemical industry. The contaminants range from chlorinated aliphatics, hexachlorocyclohexane and chlorobenzenes to chlorinated dibenzo-*p*-dioxins and dibenzofurans. Recently, two novel *Dehalococcoides* strains were enriched from the Bitterfeld area, strain DCMB5 from highly dioxin-contaminated river sediment and strain BTF08 from a groundwater well contaminated by chlorinated ethenes.
Although highly-chlorinated benzenes, phenols and dibenzo-*p*-dioxins were dechlorinated by both strains, strain DCMB5 additionally dechlorinated aromatics with fewer chloride groups. These compounds acted as electron acceptors in organohalide respiration. Strain BTF08 is the first bacterium described that can couple all dechlorination steps from tetrachloroethene to ethene to growth (1).
The genomes of both strains were sequenced and annotated. The genes encoding reductive dehalogenase homologues (*rdh*) are mostly located in two so-called high plasticity regions (2). The genomes of strains DCMB5 and BTF08 contained 23 and 20 different *rdhA* genes, respectively. Only 7 *rdhA* genes of both strains were orthologous to each other. The presence of a gene in strain DCMB5 encoding an orthologue of CbrA, the functionally characterized chlorobenzene Rdh (4), is in accordance with the remarkable property of this strain to respire chlorinated benzenes. Its high level of synthesis was shown in the proteome of cells grown with chlorinated benzenes. The genome of strain BTF08 encodes orthologues of PceA, TceA and VcrA (tetra-, trichloroethene and vinylchloride Rdhs, respectively). This demonstrates that the prevailing chloroorganic pollutants selected for *Dehalococcoides* strains with the presence of a specific set of *rdhA* genes.

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PHYP056

Enzymes of the itaconate degradation pathway in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is known to be able to grow on the media containing itaconate (methylenesuccinate) as the carbon source. The itaconate degradation pathway was resolved in the 1960[th] and involves itaconate activation to itaconyl-CoA, its hydratation to citramalyl-CoA and the cleavage of citramalyl-CoA into acetyl-CoA and pyruvate [1]. However, the corresponding genes have not been known so far. In this work we have identified a putative six-gene operon, which contains genes for a class III CoA-transferase, an (*R*)-specific enoyl-CoA hydratase and a C-C-lyase. We have proposed that these genes are involved in itaconate metabolism. To test this hypothesis, these three genes were heterologously overexpressed in *Escherichia coli*, and the correspondent recombinant proteins were purified and characterized. The results confirmed the identification of the proteins as itaconate:succinyl-CoA CoA-transferase, itaconyl-CoA hydratase, and citramalyl-CoA lyase, respectively. Furthermore, these proteins were able to convert itaconate into acetyl-CoA and pyruvate, thus approving a successful reconstruction of the itaconate degradation pathway *in vitro*. Consequently, a citramalyl-CoA lyase mutant could not utilize itaconate as a sole carbon source. Interestingly, the *P. aeruginosa*-like itaconate degradation gene cluster was found in many other species, particularly in numerous pathogens like *Brucella* spp., *Bordetella* pp. and *Burkholderia* spp. In fact, itaconate may be an important carbon source for the pathogens during infection, since it has recently been identified as a mammalian metabolite whose production is strongly induced during macrophage activation [2]. Furthermore, itaconate is a potent inhibitor of the glyoxylate cycle [3] which is important for the survival of many pathogens inside the eukaryotic host [4], and itaconate detoxification may be important for pathogenic species capable to survive in macrophages.

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PHYP057

Itaconate degradation pathway in *Yersinia pestis*

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Yersinia pestis harbors three-gene *rip* (**r**equired for **i**ntracellular **p**roliferation) operon, which is mandatory for replication in activated macrophages [1]. This operon encodes genes for a class III CoA-transferase (*ripA*), an (*R*)-specific enoyl-CoA hydratase (*ripB*) and a C-C-lyase (*ripC*). We have proposed that these genes may be responsible for the degradation of itaconate (methylenesuccinate), a recently identified mammalian metabolite whose production is strongly induced during macrophage activation [2]. Bacterial itaconate degradation pathway includes activation of itaconate to itaconyl-CoA, its hydration to citramalyl-CoA and the cleavage of citramalyl-CoA into acetyl-CoA and pyruvate [3]. To test possible involvement if *ripABC* in itaconate metabolism, we synthesized the *ripA*, *ripB* and *ripC* genes from *Y. pestis* and purified and characterized the recombinant proteins. With these three proteins we were able to reconstruct the itaconate degradation pathway *in vitro* and could assign RipABC to the proposed function, itaconate:succinyl-CoA CoA-transferase (RipA), itaconyl-CoA hydratase (RipB), and citramalyl-CoA lyase (RipC). A *Y. pestis*-like gene cluster can be found in many pathogens such as *Y. pseudotuberulosis*, *Salmonella enterica*, *Burkholderia mallei*, *B. pseudomallei*, *Mycobacterium ulcerans*, *Mycoplasma synoviae* and *Legionella longbeachae*, among others. Interestingly, the *rip*-genes were shown to be crucial for survival of *Y. pestis* and *S. enterica* in macrophages [1, 4]. Since itaconate is known to be a potent inhibitor of the glyoxylate cycle [5], and because the glyoxylate cycle is important for the survival of many pathogens inside the eukaryotic host [6], we hypothesize that its utilization through the enzymes encoded by *rip*-operon abolishes glyoxylate cycle inhibition. In addition, itaconate might serve as an excellent growth substrate for the pathogens.

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PHYP058

An *E. coli* indicator strain for biotin transport activity

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Biotin is an essential cofactor e.g. in fatty acid biosynthesis. Prokaryotes either synthesize biotin or take it up from the environment, but organisms with restricted biosynthetic capacity strictly rely on uptake. Vitamin uptake in many prokaryotes is mediated by ECF (energy-coupling-factor)-type ABC importers [1, 2]. ECF transporters consist of a substrate-specific transmembrane protein (S unit) and an energizing module composed of a conserved transmembrane protein (T unit) and ABC-ATPase domains (A units) in unknown stoichiometry. Like other S units, the biotin-specific BioY proteins bind their substrate with extremely high affinity in the (sub)nanomolar range. Questions of whether BioY functions as a transporter itself in the absence of T and A units are discussed controversially [3, 4]. In about one third of *bioY*-containing genomes annotated in the SEED database, genes for T units are not recognizable underscoring the potential role of solitary BioY as a transporter. To clarify this question we constructed an *E. coli* reporter strain. This strain lacks the *bioH* gene and thus, cannot produce the biotin precursor pimeloyl thioester. Moreover, its *yigM* gene, encoding a non-ECF-type biotin transporter [5], was deleted. The strain contains a recombinant pimeloyl:coenzyme A ligase and hence, it survives upon addition of pimelic acid which is converted to biotin. As predicted, the

mutant strain did not grow on biotin at trace concentrations. Individual BioY proteins from various sources were tested for restoration of growth on 1 nM biotin. Production of some BioY proteins was not tolerated by the cells. Individual expression of the functional *bioY* genes from *Bradyrhizobium japonicum, Oceanicola batsensis*, *Roseobacter denitrificans* and *Silicibacter pomeroyi*, however, stimulated growth on traces of biotin demonstrating uptake of the vitamin into the cells. We conclude that BioY proteins not only bind the vitamin on the cell surface but can transport it in sufficient quantities across the membrane in the absence of a cognate energizing module. This activity may depend on dimerization/oligomerization of BioY, a feature that was observed for the *Rhodobacter capsulatus* BioY during live-cell FRET analyses [6] and examination of a covalently fused dimer [7].

References: www2.hu-berlin.de/biologie/microbio/R.htm

PHYP059

Mutational analysis of the Rubber Oxygenase active site indicates participation of F317 and F301 in interaction with substrate

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RoxA is an extracellular *c*-type dihaem cytochrome secreted by *Xanthomonas* sp. during growth on polyisoprene (rubber). Purified RoxA cleaves rubber to low molecular product ODTD (12-oxo-4,8-dimethyltrideca-4,8-diene-1-al) by an unknown dioxygenase mechanism [1]. Recently, the three-dimensional RoxA structure was solved and showed two axial histidine ligands for the C-terminal haem and one axial histidine ligand for the N-terminal haem that represents the active site. The distal site of this haem is encased by a hydrophobic cavity which consists of the amino acids A251, I252, A316 as well as F301 and F317 that are in close proximity to the haem iron (≈5.9Å and 5Å, respectively).
To find experimental evidence for the involvement of both residues in catalysis six mutants were constructed (coding for RoxA variants F317A, F317L, F317Y, F317H, F317W and F301L) and over-expressed in recombinant Δ*roxA Xanthomonas* sp.. Residual clearing zone formation on opaque latex agar was found for *Xanthomonas* sp. expressing the F317L, F317A, F317H and F301L variants**.** Strains in which F317 was changed to tyrosine or tryptophan were inactive [2]. Purified RoxA muteins were characterized with respect to residual activity as well as by UVvis, circular dichroism (CD) and electron paramagnetic resonance (EPR) spectroscopy. The effects of chemical reduction of the haem centres and the influence of low molecular haem ligands such as pyridine on activity and spectral properties will be presented.Our data indicate that residue F317 and to minor extent F301 are involved in interaction with substrates.

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PHYP060

The influence of 1-acyl-sn-glycerol-3-phosphate acyl-transferase in psychrotrophy adaptation of *Serratia plymuthica* RVH1

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Psychrotrophic bacteria are an important concern for the stability and safety of refrigerated foods. The adaptations that allow low temperature growth have been mainly studied in Gram-positive bacteria. Therefore, we have initiated a study on the mechanisms of cold adaptation in *Serratia plymuthica*, a biofilm-forming Gram negative bacterium isolated from food processing line[1], as a model psychrotroph. Mutants of *S. plymuthica* RVH1 that are defective in low temperature growth (4°C) but that grow normally at 30°C were created by transposon mutagenesis. One of the mutants has the transposon insertion in the 5' upstream region of an open reading frame that was putatively identified as a 1-acyl-sn-glycerol-3 phosphate acyltransferase gene (*plsC*). PlsC transfers a fatty acid to the 2-position of lysophosphatidic acid (LPA) to form phosphatidic acid (PA), the key intermediate in the formation of most bacterial membrane phospholipids[2]. Fatty acid analysis showed a ratio of saturated to unsaturated fatty acids for the parental strain and the mutant strain, respectively, of 1.1 and 1.2 when grown at 30°C, and of 0.55 and 0.59 when grown at 10°C and 30°C. This indicated that the total ratio of saturated to unsaturated fatty acids was not significantly disturbed and that the mutant was still capable of homeoviscous adaptation. However, the mutant displayed a considerably altered ratio of C16:1 ω7c/ω6c to C18:1 ω7c/ω6c, the two major unsaturated fatty acids. When grown at 30°C this ratio was 0.5 for the mutant compared to 3.8 for the parental strain, and it shifted to 0.36 and 2.3 for both strains, respectively, when they were grown at 10°C. Genetic complementation of the mutant with a plasmid-borne *plsC* gene with an intact promoter completely restored the parental phenotype and fatty acid composition.
Our findings suggest that *plsC* expression is reduced in the mutant, and that *S. plymuthica* PlsC has a preference for incorporation of C16 fatty acids as previously suggested for *Rhizobium meliloti*[3].

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PHYP061

Growth behaviour of *pfkA pfkB pgi* triple mutants in the glycolytic pathway of *Escherichia coli* K12

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Escherichia coli K12 is a model organism in microbiology and genetics. Its genes and enzymes which govern the central metabolic Embden-Meyerhof-Parnas (glycolysis) route and pentose phosphate pathway (PPW) are well understood [1,2]. The systematic knockout of single genes has furthermore allowed to assess essential reactions in the *E. coli* central metabolism [3,4]. Combination of gene knock-outs for enzymes of the upper glycolytic pathway is presented.
Mutant strains of *E. coli* K12 W3110 which lack both genes (*pfkA*, *pfkB*) for phosphofructokinase (PFK) and the gene *pgi* for phosphoglucoisomerase were created. The triple mutant strain was analyzed (in comparison with its parent strains) for its growth behaviour in LB, various mineral salt media (MM) with both, PTS- and non-PTS substrates, and on MacConkey agar plates.
Whereas growth on LB was nearly unaffected in the *pfkA pfkB pgi* triple mutant, growth on MM with C-sources which are catabolised via the intermediate, fructose 6-P, was either abolished or severely affected. Growth on glucose was still possible, presumably via the PPW. Growth on fructose is possible as this PTS substrate is mainly catabolised via fructose 1-P and can thus circumvent the PFK step. The triple mutant was furthermore inhibited by growth on MacConkey agar plates containing bile acids. Efficiency of plating (eop) on MacConkey agar dropped for at least three orders of magnitude compared to the wild type strain. This may reflect that the lack of PGI activity can alter the composition of the lipopolysaccharide (LPS) envelope of *E. coli* which protects these gram-negative bacteria from the effects of bile acids [5]. The most severe effect could be seen on MacConkey agar plates with 0.5% of mannitol added. The eop was reduced for more than 4 orders of magnitude in comparison to the wildtype, presumably due to the combined effects of cholate sensitivity and the accumulation of fructose 6-P due to the block in PFK and PGI activities. Results on an engineering of the glycolysis pathway will be presented.

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PHYP062

Acetobacter pasteurianus DSM 3509 produces cobalamin

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A strain of acetic acid bacteria was found to have the ability to synthesize cobalamin. A preliminary genetic study of the gene of uroporphyrinogen-III synthase and a survival test indicated the ability to synthesize cobalamin. By a modified microbiological assay based on *Lactobacillus delbrueckii* spp. *lactis* DSM 20355, 4.57 ng/mL of real cobalamin and 0.75 ng/mL of analogues were detected. The product extracted and isolated in its cyanide form had the similar UV spectrum as standard cyanocobalamin and as cobalamin prodcuced by *Lactobacillus reuteri* DSM 20016. No cobalamin was detected in the fermentation broth containing 1% acetate, and less cobalamin was obtained when acetate started to be consumed.

PHYP063

The incompatible solutes creatine and guanidino-ectoine disrupt ion homeostasis

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Growth inhibitory effects of the guanidino compounds creatine and guanidino-ectoine on Gram-negative bacteria have already been demonstrated in previous studies [1], [2]. As these incompatible solutes bear high resemblance to the compatible solutes betaine and ectoine respectively, they are "by mistake" actively accumulated by compatible solute transport systems into the cytoplasm, where they cause metabolic disorder.
Although the guanidino function has been shown to render compatible solutes incompatible, the two compounds under investigation (creatine and guanidino-ectoine) appear to have different modes of action. Whereas creatine affects sodium/proton homeostasis, guanidino-ectoine does not [3].
Since (in)compatible solutes are taken up and deploy their features at elevated salinities only, where potassium homeostasis is equally important as sodium homeostasis, we investigated their potential interference with K^+ transport systems.
The influence of creatine and guanidino-ectoine on potassium fluxes following osmotic upshock was monitored *in vivo* using microelectrode ion flux estimation (MIFE) [4] and flux measurements with ion selective electrodes (FLISE) [5]. In addition, growth experiments with K^+ transport mutants and experiments with purified inside-out membrane vesicles completed the picture.
Our results demonstrated that potassium transport systems are also targeted by both guanidino-compounds, of which guanidino-ectoine plays a more prominent role as inhibitor.

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PHYP064

The Ser/Thr kinase PknB of *Staphylococcus aureus* - Coordination of cell division and cell wall biosynthesis?

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Prokaryotic signal transduction pathways regulate cellular functions in response to environmental cues and enable bacteria to react immediately to changing conditions like antibiotic stress. Besides two-component regulatory systems (TCS), one-component regulatory systems (OCS) represent one of the most abundant signaling systems in prokaryotes. These OCS include eukaryotic-like serine/threonine kinases (ESTKs) and phosphatases (ESTPs), which are increasingly recognised as important regulators of major processes such as cell wall metabolism and division, virulence/ bacterial pathogenesis and spore formation. One such ESTK/ESTP-couple has recently been identified in *Staphylococcus aureus* designated PknB/YloO [1]. The extracellular sensor part of the kinase contains three daisy-chained PASTA-domains assumed to be capable of binding peptidoglycan subunits, suggesting that PknB monitors the coordinated assembly of peptidoglycan biosynthesis and cell division. Microscopic analysis revealed the co-localisation of GFP-PknB and GFP-YycG at the septum, the site of active cell wall biosynthesis in cocci. Deletion of the PASTA-domains resulted in delocalisation of GFP-PknBΔPASTA, suggesting an interaction with cell wall precursors, thereby regulating cell wall synthesis.
To further investigate the role of PknB we analysed the interplay with the essential YycFG and the GraRS TCS on the molecular level and show phosphorylation of the response regulators YycF and GraR, implying fundamental crosstalk between the regulatory systems.
Moreover the cell division protein FtsZ was identified as a phosphorylation target of PknB, further assuming a role of the Ser/Thr kinase in cell division.

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PHYP065

Metabolism of *Cupriavidus necator* during growth and PHB production on succinic acid and fructose

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Cupriavidus necator (formerly *Ralstonia eutropha*) is well known as a producer of the biopolymer poly-hydroxybutyrate (PHB) and extensive research has been performed concerning cultivation conditions and process design as well as strain engineering for efficient PHB production [1].
In our work we focused on the metabolism of *C. necator* during the exponential growth phase and the stationary PHB production phase. Cells were grown in nitrogen limited media containing succinic acid and fructose as sole carbon sources. A maximum PHB content of 0.84 g g^{-1} CDW was observed during growth on fructose. We applied ^{13}C metabolic flux analysis (^{13}C MFA) to determine *in vivo* carbon flux distributions throughout the central metabolic pathways during exponential growth and metabolite balancing during the stationary PHB production phase also considering cellular respiration. The results from this study yielded a detailed insight into *in vivo* metabolic flux distributions on different carbon sources during exponential growth and their redirection upon stationary PHB production.

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PHYP066

Dissimilatory iron reduction in *Shewanella oneidensis* depends on the tetraheme cytochrome STC and the flavocytochrome FccA

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Terminal reduction reactions in neutrophilic dissimilatory iron reducers are localized to the cell surface. *Shewanella oneidensis* has to conduct electron transfer onto ferric iron minerals that are localized at the surface or the vicinity of the cells. Proteobacteria have to establish an electron transport chain from the cytoplasmic membrane through the periplasm and across the outer membrane in order to contact the acceptor. Past studies revealed that *c*-type cytochromes are key proteins for electron transfer in *S. oneidensis*. Electron transfer from the cytoplasmic membrane into the periplasm is catalyzed by the tetraheme cytochrome and menaquinol oxidase CymA. Transfer of respiratory electrons across the outer membrane and onto ferric iron is achieved via a tri-component complex consisting of the cytochromes MtrA and MtrC and the outer membrane β-barrel protein MtrB. Information regarding electron transfer processes that bridge the periplasm is sparse. This is most probably due to the multitude of periplasmic *c*-type cytochromes that are expressed simultaneously. Deletion mutant studies failed so far to elucidate whether - besides MtrA - any other periplasmic cytochrome is necessary for ferric iron reduction. The accentuated role of MtrA among the periplasmic cytochromes is most probably based on its interaction with MtrB and MtrC during complex formation and electron transfer. The robustness of periplasmic electron transfer was so far explained by overlapping activity of the simultaneously expressed cytochromes that prevents growth deficiencies in single deletion mutants. Nevertheless, *c*-type cytochrome based electron transfer does not necessarily have to be unspecific. An example is the electron transport chain from CymA via the small monoheme cytochrome ScyA onto the diheme peroxidase CcpA. In this study, we show that periplasmic electron transfer between CymA and MtrA specifically relies on the periplasmic tetraheme cytochrome STC (SO_2727) and the fumarate reductase FccA (SO_0970). A double deletion mutant is severely impaired in its ability to respire on iron and this inability is overcome by the spontaneous appearance of suppressor phenotypes. Here, the importance of the soluble periplasmic cytochromes FccA and STC within this electron transport chain is shown for the first time.

PHYP067

Biosynthesis of heme d_1 in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa generates energy under anaerobic growth conditions through the stepwise reduction of nitrate to molecular nitrogen during denitrification. In the second step of denitrification nitrite is converted into nitric oxide by the cytochrome cd_1 nitrite reductase (NirS). NirS contains two essential cofactors, heme *c* and heme d_1. The enzymes which are involved in the synthesis of the isobacteriochlorin heme d_1 are encoded by the *nirSMCFDLGHJEN* gene cluster.[1] In the course of heme d_1 biosynthesis siroheme is converted to 12,18-didecarboxy-siroheme by the heterotetrameric enzyme NirDLGH. In order to characterize the NirDLGH complex from *P. aeruginosa* we established an *in vivo* activity assay showing that 12,18-didecarboxy-siroheme is formed only in the presence of all four proteins. However, the heterodimeric complexes NirDL and NirGH catalyzed the conversion of siroheme to monodecarboxy-siroheme.
Interestingly, NirDLGH also possesses a regulatory functions in addition to it´s enzymatic activity. *In vivo* and *in vitro* experiments suggested that NirL and NirH bind to a DNA region upstream of the *nirJ* transcription start site thus regulating the expression of the *nirJEN* genes.
In this context, we are particularly interested in the characterization of the DNA-binding sequence, the DNA-binding domain of the proteins and the conditions that affect the regulation of the *nirJEN* genes.
Furthermore, we solved the crystal structure of the homologous NirDL protein from *Hydrogenobacter thermophilus* in complex with the substrate analogue Fe-Uroporphyrin III. The putative active site of NirDL identified as well as the potential DNA-binding domain of NirDL.

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PHYP068

Light-harvesting in Cryptophytes

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Phycobiliproteins are light-harvesting proteins, which occur in cyanobacteria, red algae and cryptophytes in addition to chlorophyll containing antenna complexes. They allow the organisms to efficiently absorb light in regions of the visible spectrum that are poorly covered by chlorophylls. Cryptophytes are extraordinary unicellular, eukaryotic algae widespread in marine and limnic waters, but largely unexplored. Their phycobiliproteins consist of an (αβ)(αβ) apo -protein covalently associated with characteristic open-chain tetrapyrroles, which act as light absorbing chromophores. Cryptophytes employ six different chromophores: phycocyanobilin (PCB), phycoerythrobilin (PEB) - and quite unusual - 15,16-dihydrobiliverdin (15,16-DHBV), mesobiliverdin (MBV), bilin 584 and bilin 618 for light-harvesting.
The biosynthetic pathway of open-chain tetrapyrroles and their attachment to the apo-protein is entirely unknown in cryptophytes. The model organism *Guillardia theta* uses the phycobiliprotein PE545, which is associated with the chromophores 15,16-DHBV and PEB. Interestingly, 15,16-DHBV is only described to occur as an intermediate of PEB biosynthesis in cyanobacteria and cyanophages but not as a bound chromophore. This raises the question of elucidating the chromophore biosynthesis and attachment in *G. theta*.
Currently, the enzymatic activities of putative bilin biosynthesis enzymes are analyzed. First results showed that a heme oxygenase (GtHO) is able to cleave heme yielding the open-chain tetrapyrrole biliverdin IXα. Furthermore, a bilin reductase (GtPebB) reducing 15,16-DHBV to PEB was identified, which will be further biochemically investigated. Due to aggregation of the protein during purification, the characterization of a second putative bilin reductase is more difficult and requires further experimentation. Finally, we are investigating the involvement of phycobiliprotein lyases in the posttranslational modification of the apo-phycobiliprotein with the chromophores PEB and particularily 15,16 DHBV.

PHYP069

The role of granulose in the obligate anaerobe *Clostridium acetobutylicum*

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The transition phase of growth of the Gram-positive, endospore forming anaerobe *Clostridium acetobutylicum* is characterized by several morphological changes. When entering the stationary phase swollen and cigar shaped cells, termed clostridial stages, are formed. In this type of cells a polymeric glycogen-like carbohydrate is accumulated in the form of granules, called granulose. This glucose-polymer is defined as an amylopectin-like structure and only slightly branched (98% α-1.4-linkages and 2% of α-1.6-linkages). Granulose is considered to store energy- and carbon, and hitherto believed to be necessary as a prerequisite for sporulation.
We generated a granulose defect mutant by inactivating (ClosTron® technology) the single glycogen synthase (GlgA) annotated in the genome of *C. acetobutylicum*. The mutant strains showed no differences in growth rate, fermentation pattern and, surprisingly, sporulation, when compared to the wild-type strain, even thought it exhibited a granulose defect phenotype. This raised questions regarding the importance and function of the granulose in the lifestyle of *C. acetobutylicum*. Therefore, starvation experiments were performed in order to investigate the importance of this glucose-polymer under periods of nutrient depletion, usually affecting soil bacteria. The strain was able to be complemented for its defective phenotype by plasmid-based *glgA* expression.
The results demonstrate that sporulation and the cell differentiation observed during *C. acetobutylicum* sporulation are independent of granulose accumulation. Furthermore, new insights concerning the importance of granulose for survival and persistence of *C. acetobutylicum* will be presented.

PHYP070

Compatible solutes of the acidophilic *Acidiphilium cryptum* JF-5 and heterologous expression of its *ectABCD* gene cluster

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In order to maintain osmotic equilibrium (under osmotic stress conditions) a broad range of microorganisms accumulate low-molecular organic compounds, the so-called compatible solutes [1]. The acidophilic α-Proteobacterium *Acidiphilium cryptum* JF-5 was first isolated from allegedly pure cultures of *Thiobacillus ferrooxidans* [2]. It is a facultative aerobe, grows in lean organic media between pH 1.9 and 5.9 and has so far not been known to tolerate increased salinity, although a hydroxyectoine gene cluster had been annotated [3].
We were able to show that *A. cryptum* is capable of synthesizing the compatible solutes ectoine, hydroxyectoine and trehalose, but against expectations the bacterium showed a relatively low NaCl tolerance (approx. 5 %). As another unusual feature its main solutes, hydroxyectoine and trehalose, are produced concomitantly, both increasing with salinity.
Furthermore we succeeded with the heterologous expression of the *A. cryptum ectABCD* gene cluster in *Escherichia coli*, which encodes the enzymes involved in ectoine and hydroxyectoine biosynthesis from the precursor L-aspartate. Hence we could for the first time prove metabolic functionality of the *A. cryptum ectABCD* gene cluster.
In addition we present information on enzyme activity of *A. cryptum* ectoine synthase (EctC) *in vitro*. EctC catalyses the intramolecular condensation of ADABA (Nγ-acetyl-diaminobutyric acid) to ectoine. In comparison to EctC from the moderate halophile *Halomonas elongata*, which requires NaCl for optimal enzyme activity [4], the *A. cryptum* enzyme exhibits the highest activity in the absence of NaCl.
Based on these results we conclude that the cytoplasmic environment in *A. cryptum* is different to that of *H. elongata* and that its hydroxyectoine gene cluster, besides salt adaptation, may serve additional functions.

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PHYP071

Degradation of raffinose by a novel strain of *Pseudomonas*

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A Gram-negative, chemoorganotrophic, cyanide forming, flagellated, rod-shaped bacterium (strain CCOS191) was isolated from soil. CCOS191 was phylogentically characterized applying 16S rRNA and MLSA (multilocus sequence analysis) gene sequence similarity as well as by DNA-DNA hybridization and revealed to be a novel species within the genus *Pseudomonas*. Closest relatives were *P. mosselii* and *P. entomophila*. Carbon source utilization testing with BiOLOG Phenotype MicroArrays and subsequent comparison with ten related *Pseudomonas* species by principal component analysis showed a very special metabolic feature - which makes CCOS191 unique - namely the utilization of raffinose, a trisaccharide consisting of glucose, fructose, and galactose. Although earlier very few pseudomonads have been described as being able to utilize raffinose as sole carbon source, most of the strains have been reclassified and renamed. Today, only a few strains of *P. syringae* - but not all - can grow on raffinose as sole carbon source. For the microbial degradation of raffinose, the activity of alpha-galactosidase is required. Only very few *Pseudomonas* species are known to produce this enzyme, e.g., *Pseudomonas atlantica* (a marine bacterium, reclassified as *Alteromonas atlantica* and later as *Pseudoalteromonas atlantica*). However, *P. atlantica* was described as not being able to utilize raffinose, although alpha-galactosidase activity was observed. In our case, the closest phylogenetic relatives of CCOS191 (*P. mosselii* and *P. entomophila*) were not able to grow at all on raffinose. In summary, genotypic analysis in combination with physiological testing clearly demonstrated that strain CCOS191 represents a novel species within the genus *Pseudomonas* for which the name "*Pseudomonas raffinosivorans*" sp. nov. is proposed.

PHYP072

The Rnf complex- a novel ion pump of the anaerobic respiration

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Low-potential ferredoxin (E_0` ≈ -500 mV) is a common electron carrier in anaerobes (1). It is used to drive endergonic reactions and it is also used as electron donor for a membrane-bound enzyme, the ferredoxin:NAD^+ oxidoreductase, that couples exergonic electron transport from ferredoxin to NAD^+ to the translocation of Na^+ from the cytoplasm to the medium. This enzyme is hypothized to be encoded by the *rnf* genes that are present in many bacterial and a few archaeal species (2). Here we present the central role of the Rnf complex in the metabolism of two phylogenetically very different organisms, the bacterium *Acetobacterium woodii* and the archaeon *Methanosarcina acetivorans*. We will demonstrate by biochemical and genetic analyses that the Rnf complex functions as a primary, electrogenic Na^+ pump in both of these organisms accepting low potential electrons from ferredoxin. In *A. woodii* the electrons are transferred from ferredoxin to NAD^+ (3), whereas in *M. acetivorans* Rnf is part of a more complex electron transport chain. Electrons from ferredoxin are transferred by the Rnf complex to the membrane-bound methanophenazine, a quinol-related electron carrier. Finally these electrons are channelled to heterodisulfide by the heterodisulfide reductase (4).
Our studies demonstrate that the Rnf complex is a key ion-translocating enzyme complex in anaerobic metabolism and broaden the knowledge of electron transport chains fueled by low potential electrons.

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PHYP073

Pathway of autotrophic CO_2 fixation in *Nitrosopumilus maritimus*

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Ammonia-oxidizing Archaea is a recently described prokaryotic group which contributes significantly to global ammonia and carbon cycling [1]. Genome analyses of these organisms suggest that they belong to a novel archaeal phylum Thaumarchaeota [2]. Representatives of this phylum are highly abundant in terrestrial and marine environments, accounting for up to 20% of the oceanic picoplankton [3]. The first member of this group isolated in pure culture is *Nitrosopumilus maritimus* [4]. While its genome sequence suggests autotrophic growth via the 3-hydroxypropionate/4-hydroxybutyrate cycle [5, 6], biochemical evidence was still missing. In our work we were able to detect the activities of key enzymes of this pathway in *N. maritimus* cell extracts. Furthermore, the cell extracts catalyzed the conversion of 3-hydroxypropionate into propionyl-CoA as well as the conversion of 4-hydroxybutyrate into acetyl-CoA. Several genes which encode enzymes putatively catalyzing the specific reactions of the cycle were heterologously expressed in *Escherichia coli*, and the corresponding proteins were purified and characterized. Taken together, the results of our work give strong evidence for the functioning of a 3-hydroxypropionate/4-hydroxybutyrate cycle in *N. maritimus*. The possible scenarios of the emergence of this cycle in Thaumarchaeota will be discussed.

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PHYP074

Analysis of the carbon flow in response to iron supply in *Clostridium acetobutylicum*

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Due to its capability to produce the bulk chemicals acetone and butanol, the obligate anaerobe *Clostridium acetobutylicum* has a longstanding history in the field of the commercial production of solvents. In recent years, researchers and industry have regained interest in the complex biphasic metabolism of this organism, to improve its butanol productivity [1]. While the overall network is well understood, little is known about the bottlenecks directing the flux from glucose to butanol, as well as their respective dynamical regulation. One of such bottlenecks is thought to be linked to the availability of iron [2], since it is a major co-factor of many enzymes, involved in the main metabolism of this bacterium.
Here, we present the results of a study systematically analysing the effects of iron limitation on *C. acetobutylicum*, grown in continuous chemostat cultures. Thereby, the main focus point lies on the altered electron and carbon flow in comparison to phosphate limited cultures. To better understand and interpret the data, currently an existing metabolic network model is extended and adjusted.
This study shows, that iron limitation alters the production of virtually every measured product significantly, indicating the deep reaching impact of a shortage in this crucial micro nutrient. Thereby, the results vary for cultures grown at pH levels which promote the production of acids as compared to cultures grown at solvent favouring pH levels.

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PHYP075

Cholate degradation in actinobacterium *Dietzia* sp. strain Chol2: An unexplored pathway for bacterial steroid degradation

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Steroids are ubiquitous natural compounds with diverse functions in eukaryotic organisms. Bacteria from different phylogenetic groups are able to degrade steroid compounds for utilizing them as sole carbon and energy sources. The only well-described pathway for aerobic degradation of steroid compounds by bacteria is the so-called 9,10-seco pathway[1]. For investigating whether the ability of degrading bile salts is widespread among environmental bacteria, enrichment cultures with the bile salt cholate as substrate and sludge from different sources as inoculum were set up. A number of strains were isolated that could grow with cholate as carbon and energy source. All strains degraded cholate via the 9,10-seco pathway except one the steroid-degrading actinobacterium, *Dietzia* sp. strain Chol2. Growth experiments showed that strain Chol2 was not able to grow with the steroid compounds, which are characteristic intermediates of the 9,10-seco pathway indicating that a different pathway for steroid degradation is operative in strain Chol2. To investigate this pathway experiments with cell suspensions and cell extracts were performed. These experiments showed that cholate was converted into 12α-hydroxy-3-oxo-4,6-choldienoic acid (HOCDA) via 3-ketocholate and Δ^4-3-ketocholate. HOCDA was further converted into 3,12-dioxo-4,6-choldienoic acid (DOCDA). In vitro, this reaction was strictly dependent on NAD^+ as electron acceptor and could only proceed after CoA-activation of the HOCDA. Strain Chol2 was able to transform but not to grow with deoxycholate and chenodeoxycholate. These results showed that cholate degradation in strain Chol2 proceeds via an unexplored pathway that is initiated by elimination of the hydroxyl group at C7 and by oxidation of the hydroxyl group at C12. Thus, this pathway differs substantially from the 9,10-seco pathway showing that the biochemical diversity of bacterial steroid degradation has been underestimated so far. The further elucidation of this pathway is currently on the way in our laboratory.

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PHYP076

Function of cyanobacterial phycobiliprotein lyases in *Prochlorococcus marinus* SS120

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Phycobiliproteins (PBP) are widely distributed in cyanobacteria. The PBPs are assembled to larger structures called phycobilisomes which efficiently increase light harvesting capacity during the photosynthesis. The light harvesting properties of PBPs are mediated through phycobilins, open-chain tetrapyrrole molecules. Bilins are attached to apo-PBPs with the help of phycobiliprotein lyases. In addition to their function during attachment of the bilin to the apo-PBP, some lyases possess additional isomerase activity, resulting in the formation of new bilins. So far such mechanisms are known for the formation of phycoviolobilin (PVB) from phycocyanobilin (PCB) and for phycourobilin (PUB) from phycoerythrobilin (PEB). In this work we biochemically characterise PBP-lyases from the extraordinary cyanobacterium Prochlorococcus marinus SS120. This low-light adapted ecotype is one of the smallest organisms known to perform oxygenic photosynthesis. It lacks phycobilisomes and mainly harvests light through chlorophyll-binding proteins. However, besides possessing a single PBP, genes encoding six putative PBP-lyases and the biosynthetic machinery for PCB and PEB are present in the genome. Interestingly, enrichment experiments demonstrated that the PBP from P. marinus SS120 is rich in PUB. Aim of this work is to reconstruct the complete PBP-biosynthesis of P. marinus SS120 in E. coli and elucidate the biosynthesis and/or attachment of PUB. In addition, selected PBP-lyases will be biochemically characterised. Here we show the first results of coexpression experiments of the PBP-biosynthesis and assembly in E. coli.

PHYP077

Molecular determination of the interaction surface of the carbon flux regulator Crh and the methylglyoxal synthase MgsA in Bacillus subtilis

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In Bacillus subtilis, the phosphoenolpyruvate phosphotransferase system plays a central role in carbohydrate uptake. Besides uptake of different sugars the components of the PTS exert regulatory functions. This also accounts for the paraloque of HPr, Crh. As a unique function of Crh in B. subtilis, it regulates the flux through the harmful methylglyoxal bypass of glycolysis. This regulation involves inhibition of the key enzyme MgsA by direct interaction with the non-phosphorylated Crh protein (1).
Phosphorylation and dephosphorylation of Crh depends on the metabolic state of the cell. In the presence of preferred substrates such as glucose, the FBP level increases leading to the formation of Crh~P. Hence MgsA is released from inhibition resulting in activation of the methylglyoxal bypass. (2).
To further characterize the interaction surface between Crh and MgsA we conducted a genetic screen to search for MgsA variants exhibiting decreased binding of Crh. We repeatedly isolated several mutants carrying exchanges in the N-terminus and putative active center of MgsA. Surprisingly all of the analyzed mutants are functionally active indicating that the interaction with Crh does not interfere with the enzymatic activity of MgsA.
To proof the biological significance of these amino acid residues in the putative interaction surface of Crh and MgsA, we searched for compensatory mutants that restore the binding of Crh to the respective MgsA variants. Corresponding Crh variants, harboring mutations within the α-helices 1 and 2 could be identified. This confirms that the amino acids in the N-terminus of MgsA directly participate in the interaction with Crh.

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PHYP078

Studies on suspect persister genes and glucose metabolism of antibiotic tolerant *S. aureus* cells

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Persister cells are phenotypic variants within isogenic bacterial cultures. They form a subpopulation of drug-tolerant cells clinically relevant regarding recurrent or chronic infections. Staphylococcal persisters have been first described in 1944, but molecular mechanisms and genetics underlying this phenomenon are still not well understood. Since persister genes in other bacteria include stress-response factors, we analyzed a number of S. aureus mutants for altered levels of survivors upon antibiotic treatment. DSM20231 derived mutants ΔclpB, ΔclpC, ΔclpB/C and ΔlexA, Δagr, ΔrelP and ΔrelQ of strain HG001 were treated with super-MICs of daptomycin and life-counts were monitored over time. Compared to the wildtype, ΔclpB/C in exponential growth phase showed the lowest survival rate followed byΔclpB and ΔclpC. By contrast, Δagr appeared more drug tolerant than the other HG001 strains in exponential growth, but more susceptible in stationary phase under tested conditions. Genes katA and sodA involved in reactive oxygen species detoxification and smpB relevant in ribosome rescue are also under investigation in regard to persister formation. Preliminary experiments on metabolic activity of daptomycin treated S. aureus persisters indicate active glucose metabolism, including the incorporation of glucose derived 13C isotopes into de novo synthesized amino acids. Further experiments to quantify glucose consumption and uptake and to delineate metabolic pathways of respective cells are under way.

PHYP079

S. aureus persister gene identification: A gene-bank overexpression approach

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Bacterial persisters are slowly or non-growing cells among an isogenic bacterial culture. In contrast to their normally growing siblings persisters are tolerant to otherwise inhibitory concentrations of single or multiple antimicrobials. Little is known about the associated molecular mechanisms of Staphylococcus persisters. We aimed to identify genes affecting persister levels by expressing genomic library fragments (1-3.5 kbp) of S. aureus SA113 from a tet-inducible plasmid and challenging respective cells during exponential growth with super-MICs of the antibiotics daptomycin, tobramycin or ciprofloxacin. Killing curves obtained from monitoring life counts over time served as the retrospective readout for drug tolerance. Several clones with altered persister levels were identified. Three interesting candidates encoded a truncated ribosomal protein rpsH, an ABC-transporter permease or an antisense-fragment of a putative cell wall anchored hypothetical protein. SA113 cells overexpressing ABC-transporter permease were more tolerant to ciprofloxacin than wildtype cells but lost their tolerance towards tobramycin treatment. Tolerance was also decreased after expressing antisense-fragment of a hypothetical cell wall anchored protein and treatment with tobramycin whereas tolerance to ciprofloxacin was increased. The most striking persister phenotype observed with either tobramycin or ciprofloxacin challenge was observed upon overexpression of truncated rpsH which showed a dramatic increase in tolerance. Approaches to reveal causative relationships between the identified genomic fragments and the persister phenotype are in progress and so is the identification of additional factors leading to drug tolerance of S. aureus.

PHYP080

Complex iron-sulfur molybdoenzymes (CISM): Modular composition of enzymes and multiple functions in respiratory processes - a route for differentiation

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Complex iron-sulfur molybdoenzymes - previously described as molybdoenzymes of the dimethyl sulphoxide reductase (DMSOR) family - are a widespread class of proteins and have essential functions in energy conserving metabolisms of *Bacteria* and *Archeae* [1]. They are involved in carbon, nitrogen, arsenic, selenium and sulfur metabolism. CISM are made up of modules. Normally they compose of a catalytic Mo-bis-MGD-containing subunit with one [4Fe-4S] cluster, an electron-transfer subunit with four [4Fe-4S] clusters and a quinone/quinol reactive membrane-subunit. In some respiratory CISM the catalytic subunits interact with soluble or membrane-bound c-type cytochromes. CISM are very diverse in their enzymatic reactions and respiratory functions but they are not so diverse in their primary protein structures within their homologous, modular subunits. Based on amino acid sequences homologies/similarities of the corresponding subunits among themselves an unambiguous assignment of specific enzyme functions to the molybdenum-containing enzymes is often not possible. The quantity of false annotated CISM in genome data bases reflects that problem. So in future several information of a CISM should be regarded together for more secured annotations. Besides the classic biochemical identifications of key enzyme-activities the following data should be included: genetic organizations of subunit encoding genes within the gene-clusters (in combination with additional genes for chaperones and/or two-component signal transducing systems), additional bioinformatics analysis with the focus on the active sites of the enzymes (Molybdenum-ion ligation by cysteine- or serine-residue), cofactor-ligations of iron-sulfur-centers by cysteine-clusters and structural predictions of quinone/quinol reactive membrane-subunits including functional amino acid residues. All information together should have to point out a better understanding of this kind of enzymes and should bring forth the annotation of molybdoenzymes of the CISM family in future.
CISM from *Wolinella succinogenes* will be presented as examples [2] and will be integrated in a primary flowchart for CISM annotation.

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PHYP081

Proteome analysis in *Schizosaccharomyces pombe*: Amino acid biosynthesis and membrane composition as tragets for improved protein secretion

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Protein secretion in yeast is a complex process and its efficiency depends on a variety of parameters. To investigate the influence at the various steps of protein secretion and the overall proteomic response of the cell to the burden of protein production, we constructed a set of Schizosaccharomyces pombe strains producing maltase (1) with different yields. Proteome analysis revealed changes in chaperon levels as well as proteins involved in the secretory transport machinery and proteins controlling transcription and translation. We found an unexpectedly high amount of changes in enzyme levels of the central carbon metabolism and a significant up-regulation of several amino acid biosynthesis genes. Analysis of the average amino acid composition of the cellular protein of S. pombe and comparison with our model protein pointed towards an over-representation of the corresponding amino acids in maltase compared to the composition of the cellular protein. Additional feeding of these amino acids resulted in an increase in protein secretion by a factor of 1.5. Ergosterol biosynthesis was identified as a second target and addition of Fluconazol or Amphotericin B caused a significant decrease in ergosterol levels while protein secretion could be further increased by a factor of 2.1. Thus we could show that the central carbon metabolism can be one bottleneck of protein secretion in yeast and proteome analysis can be used to get targets that allow an efficient increase of protein secretion in the yeast Schizosaccharomyces pombe.

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PHYP082

Fructose Utilization of *Clostridium acetobutylicum*

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As a member of the saccharolytic Clostridia, a variety of different carbohydrates as carbon and energy source like glucose, fructose or xylose can be utilized by *Clostridium acetobutylicum*. Generally, carbohydrates were taken up via three types of transporters: symporter, ATP-binding cassette (ABC) transporter and phosphotransferase systems (PTS). For the uptake of hexoses, hexitols and disaccharides thirteen PTS have been identified in *C. acetobutylicum*.[1]
Although fructose is an important sugar in food industry and part of technical substrates such as molasses, up to now fructose metabolism gained only little attention. The uptake and catabolism of fructose in *C. acetobutylicum* was investigated, to get new insights in its physiology.
For the transport of fructose into the cell of *C. acetobutylicum* there are mainly three PTS responsible.[2] The apparent primary fructose transport system is encoded by a polycistronic operon (*cac0231-cac0234*) including a putative DeoR-type transcriptional regulator (FruR, CAC0231), a 1-phosphofructokinase (FruB, CAC0232), a PTS IIA (FruC, CAC0233) and a PTS IIBC (FruD, CAC0234). Thus the other two PTS (CAP0066-CAP0068 and CAC1457-CAC1460) seem to play a secondary role in fructose uptake.
Here, we elucidate the importance of the fructose (*fru*) operon CAC0231-CAC0234 for the growth of *C. acetobutylicum* on fructose as sole carbon source.

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[2]Servinsky et al., 2010. *Microbiology* 156:: 3478-3491.

PHYP083

Impact of silver nanoparticles on bacterial biofilms

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Engineered silver nanoparticles have been used in different commercial products like medicine, textiles or food industry. Due to wide use in this products, environmental concentration of silver nanoparticles (nAg) e.g. in the river Rhein is donated in a range from 4 – 320 ng/l in the water column and from 0,04 – 14 mg/l in the sediment [1].
Monospecies biofilms (A .citratiphilum) were exposed to silver nanoparticles (citrate stabilized) in different concentrations [300 µg/l – 2400 µg/l] with different sizes (10 nm, 40 nm, 70 nm) for 21 h. To obtain information about cell's viability and biofilm architecture fluorescent dyes (LIVE/DEAD® BacLight™ Bacterial Viability Kit) and epifluorescence digital imaging was used. The results indicate that there is no toxic effect on cells of A. citratiphilum by exposure of nAg in the size of 10 - 70 nm and given concentrations although alterations in biofilm architecture appeared. Furthermore no differences in protein expression concerning the different treated samples could determined by SDS-PAGE.
To understand the impact of nAg on the nanomechanical properties of bacterial cell surface and biofilm atomic force microscopy (AFM) was applied. Biofilms with and without nAg (40 nm, 70 nm), [600 µg/l] were grown for different time periods (8/12/16/20/24 h). By quantifying the AFM tip-cell interactions like dissipation, stickiness (adhesion), indentation depth (deformation) and roughness over the various sections of bacterial cell surface and biofilm surface differences in nAg-treated and untreated biofilms were measured.

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PHYP084

Quantitative measurements of heterogeneous life-cycle dynamics in *Bacillus subtilis*

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Non-genetic heterogeneity is increasingly recognized as an important survival factor for bacterial populations and several strategies how to generate heterogeneity have been described. However, for *B. subtilis* sporulation different studies have reached different conclusions regarding the underlying mechanism for generating heterogeneity. Here, we report the development of a microscopy-based life-cycle assay and quantitatively measure life-cycle progression under different nutrient conditions. As expected, germination and growth rates increase with increasing nutrient levels. Surprisingly however, the sporulation behavior changes not only quantitatively but also qualitatively with nutrient conditions. For populations on low nutrient gels heterogeneity during sporulation was maintained via slow, broadly asynchronous cell differentiation. In contrast, for populations on high nutrient gels sporulation proceeds in two temporally separated waves. This suggests that - depending on environmental conditions - *Bacillus subtilis* might employ different strategies to maintain a heterogeneous population, which might also provide an explanation resolving the conflicting earlier reports.

PHYP085

Ribosomal protein L25 and its potential role in cell growth and ribosome stability under thermal stress

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The stability of ribosomes is supposed to be one important factor in growth and survival of microorganisms under thermal stress [e.g. Lee 2002]. In this context the ribosomal protein L25 caught our attention since it was shown to be important for the temperature limit of *E. coli* growth but not essential for ribosome assembly [Korepanov 2007]. Besides sharing a conserved rRNA binding domain some L25 sequences from different species are significantly elongated by an equally well conserved domain homologous to the *Bacillus subtilis* CTC general stress protein [Volker 1994].
In this project we complemented a L25 deficient *E. coli* strain with both the native protein and its CTC-domain bearing counterpart from the halophile *Halomonas elongata*. We show that a reconstitution of the growth phenotype is possible by expression of L25, but only to a certain extent with the *H. elongata* protein. Furthermore we demonstrate that L25 has a significant influence on ribosome thermal stability with and without additional salt stress.

Korepanov 2007. J Mol Biol 366, p1199
Lee 2002. Appl Environ Microbiol 68, p5379
Volker 1994. Microbiology, 140, p741

PHYP086

Investigation of proton pumps in *Staphylococcus aureus*

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In many aerobic and facultative aerobic microorganisms the first complex of the respiratory chain is the NADH-quinone oxidoreductase. In *Escherichia coli* the NADH-dehydrogenase complex I is well studied and is composed of > 12 proteins and genes (*nuoA-N*). In *Staphylococcus* a corresponding gene cluster is absent and therefore the question remains how the respiratory proton/ion translocation is mediated in staphylococci. We have identified a hypothetical protein in *Staphylococcus aureus* showing relatively high sequence similarity to the main proton-translocating subunit of NADH-quinone oxidoreductase in *E. coli* and many other prokaryotes.
The hypothetical homolog in *S. aureus* is a membrane protein and apparently organised in an operon comprising three genes. The first gene and the complete operon were deleted and phenotypically analysed. Both deletions showed a severe growth defect, a small colony variant phenotype, and decreased membrane potential. The results suggest that this operon plays a crucial role in energy metabolism and cellular fitness.

PHYP087

In vitro evolution of *Paracoccus denitrificans*

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Laboratory evolution studies have been mainly performed with *Escherichia coli* under aerobic conditions, focusing on the adaptation with respect to the conversion of the carbon source. Several studies have shown that microorganisms diverge into subpopulations using either the supplied carbon source or metabolites excreted by one subpopulation. Though, cross-feeding is not only interesting in terms of carbon metabolism, but also in anaerobic nitrate respiration. Nitrate is reduced in four reaction steps to dinitrogen via nitrite, nitric oxide and nitrous oxide, with at least three steps having the potential to be performed by a different subpopulation. In this study we aim to get insights into the driving forces that are responsible for specialization and the evolution of subpopulations feeding on different nutrients.
We performed *in vitro* evolution experiments in chemostats with *Paracoccus denitrificans*, a model denitrifying organism, under acetate (electron donor) or nitrate (electron acceptor) limiting conditions. Production of metabolites of nitrate and the utilization of the carbon source were monitored. In addition, biomass was analyzed for yield and assimilated carbon and nitrogen. Throughout the experiment we observed changes in morphology and physiology of the cells.
The metagenome and metatranscriptome will be analyzed to gain knowledge about how many subpopulations evolved and how they differ from each other. We will focus on mutations affecting the acetate and nitrate utilization pathways. Further, the evolved cultures will be compared with the ancestral organism on genetic, transcriptomic, proteomic and metabolic level. Knowledge about specialization in these pure cultures is an important step towards understanding the complex microbial behavior in natural nitrate reducing communities.

PHYP088

Physiological and molecular adaptations of *Lactococcus lactis* to zero-growth conditions

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Due to variable-nutrient availability in natural environments, microorganisms live in a feast or famine existence, with famine as the prevalent state (1). When conditions are favourable, carbon and energy sources are first consumed for growth-associated processes (2). Under conditions with limited nutrient supply, most metabolic energy is diverted to maintenance instead of growth. Many industrial fermentation processes also involve long periods of low availability of nutrients for microorganisms. An example is the process of cheese ripening, in which lactic acid bacteria in the cheese matrix are depleted from fermentable substrates (3). Survival under these conditions requires adaptations of cellular metabolism, and coincides with extremely slow or no-growth of the microorganisms. Zero-growth is defined as a metabolically active, non-growing state of a microorganism in which product-formation capability is maintained and thereby is fundamentally different from stationary phase or starvation.
Retentostat cultivation system has been designed to simulate zero-growth conditions. Retentostat cultivation is a modification of chemostat cultivation in which the growth limiting carbon source is fed at a constant rate, while biomass is retained in the bioreactor by a retention filter-probe in the effluent line. Extended retentostat cultivation leads to growth rates that approximate zero while the rate of energy transduction equals the maintenance energy requirement (4, 5).
The aim of this study is to examine quantitative physiological and molecular responses of the plant-isolated *Lactococcus lactis* KF147 in zero-growth conditions. After cultivating *L. lactis* at extremely low growth rates in glucose-limited retentostat conditions, cell morphology and physiology, metabolic profile, and robustness of the strain were investigated. Furthermore, extensive retentostat cultivation enabled the calculation of energy requirements, substrate- and energy-related maintenance coefficients and biomass yields, under non-growing conditions. The biomass concentration was accurately predicted by a maintenance coefficient from retentostat. Finally, transcription profiling of the strain was performed during retentostat cultivation.

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PHYP089

The simplest signal: protons as second messengers controlling cell division rate and more

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All organisms have evolved to cope with changes in environmental conditions, ensuring the optimal combination of proliferation and survival. Using the yeast deletion collection to study the acquisition of tolerance towards lethal conditions upon pre-exposure to mild stresses, we found an inverse correlation between mutant growth rate and stress survival. Stress resistance and acquired stress tolerance in *S. cerevisiae* are governed by a combination of stress-specific and general processes. The reduction of growth rate, irrespective of the cause of this reduction, leads to redistribution of resources toward stress tolerance functions, thus preparing the cells for impending change. This coupling of the two objective functions of optimal growth and optimal robustness appears to be anchored at the heart of metabolism.
So how is growth rate controlled? We recently identified a new aspect of this control, namely intracellular pH (pH_i). The proton is the most coupled metabolite in the metabolic network. Its concentration is usually assumed to be constant, but in fact pH_i is highly dynamic. pH_i is robust towards gene deletion, and its control requires not only vacuolar proton pumps, but also strongly relies on mitochondrial function. We show that pHi quantitatively controls yeast growth rate, likely as a second messenger: The control could be alleviated by deletion of single ORFs, revealing that protons function as a signal controlling growth.

PPAV001

RND-type multidrug efflux pumps of the fire blight pathogen *Erwinia amylovora*

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Erwinia amylovora, a plant pathogenic member of the *Enterobacteriaceae*, causes fire blight on rosaceous plants, with economic importance on apple and pear trees. Fire blight is one of the most devastating plant diseases in Germany, especially apple orchards in the South are severely affected by this existence-threatening disease. The commercial implications of this plant disease are aggravated by the limited effectiveness of current control measures.
Multidrug efflux pumps are highly conserved in bacteria and show a significant impact on antimicrobial resistance. Resistance-nodulation-division (RND) family transporters typically operate as a tripartite system including the active transporter together with a periplasmic membrane fusion protein and an outer membrane channel and are known to mediate multidrug efflux.
The RND-type efflux pump AcrAB was shown to be a major pathogenicity factor in *E. amylovora*. It was previously shown that this efflux system confers resistance to a broad range of structurally unrelated compounds including antibiotics, dyes and plant-derived antimicrobials. Moreover, an *acrB*-deficient mutant showed a dramatically reduced virulence on apple rootstocks.
Beside AcrAB, three additional RND-type pumps are present in the annotated genome of *E. amylovora*. AcrD, a homolog of AcrB, shows high homology to the aminoglycoside efflux pump AcrD of *E. coli*. Aminoglycosides are highly hydrophilic and positively charged molecules and some function as antibiotics including gentamicin, kanamycin and streptomycin. The efflux systems MdtABC and YegMNO of *E. amylovora* show significant sequence homology to the multidrug transporter MdtABC of *E. coli*. Proposed substrates are flavonoids which are polyphenolic secondary metabolites synthesized by plants.
In order to determine the impact of the different RND-type efflux pumps on antimicrobial resistance, we generated transporter-deficient mutants in *E. amylovora*. Furthermore, the contribution of these efflux pumps to virulence was evaluated on apple plants by means of the development of disease symptoms and by monitoring the establishment of a bacterial populations.

PPAV002

Elucidating the translocation of the fungal effector Cmu1

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A successful colonization of plants by pathogens requires active effector-mediated suppression of defense responses. Previously we could show that the biotrophic fungus *Ustilago maydis* secretes Cmu1, an active chorismate mutase, upon infection of its host plant maize. The effector Cmu1 translocates into the host cytoplasm and interferes with biosynthesis of salicylic acid, an important plant defense hormone. Here we report about novel insights into the translocation and structure of Cmu1. Secreted chorismate mutases existing in several other fungi were tested for their ability to complement the virulence phenotype of *cmu1* deletion strains. Comparative studies combined with mutational analyses allowed to map a specific motif essential for translocation of Cmu1 across the host plasma membrane. Cmu1 proteins in which the motif was mutated retained chorismate mutase activity, demonstrating that the overall structure of the mutant protein is not disturbed. We will also describe our attempts to identify the host-derived factors needed for Cmu1-effector translocation.

Furthermore we present the 3-D structure of Cmu1 that allows comparing the structure of the secreted chorismate mutase with previously determined structures of non-secreted enzymes.

PPAV003

Mapping functional domains of a secreted effector of *Sporisorium reilianum*

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The biotrophic smut fungus *Sporisorium reilianum* manipulates the architecture of the inflorescence of maize (*Zea mays*). Manipulation of the inflorescence includes the formation of spores or phyllody, as well as the formation of multiple cobs per branch. We showed that the formation of multiple cobs per branch can be abolished when deleting a secreted effector protein of *S. reilianum*. This effector was named Suppressor of apical dominance1 (Sad1). To shed light on the function of Sad1, we use protein-protein interaction studies as well as domain analysis.
Bioinformatic analysis showed several phosphorylation and ubiquitination sites. We could show that Sad1 is able to interact with a lot of plant originated proteins including nuclear proteins, ubiquitin ligases and kinases. To map the domains that are responsible for those interactions, a set of 13 truncated versions of Sad1 were created, each missing 12 amino acids. An interaction screen with the previously identified interaction partners revealed that the C-terminus of Sad1 interacts with most proteins. The truncated versions of Sad1 are currently tested for functionality by maize infection with recombinant *S. reilianum* strains. So far, we could show that truncations at the N-terminus of Sad1 abolish Sad1 function. These analyses will reveal, whether protein-protein interactions are important and if so, which plant interactors effectuate the function of Sad1 in suppressing apical dominance in maize.

PPAV004

Profiling of quorum sensing response and biocontrol potential of endophytic bacteria harbored in *Cannabis sativa* L.

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Cannabis sativa L. is a medicinally important plant of the Cannabaceae family containing cannabinoids as one of the major secondary metabolites. Although the plant is mainly regarded as the drug of abuse, cannabinoids are known to possess various therapeutic efficacies including analgesic, anti-spasmodic, anti-tremor, anti-inflammatory, neuro-protective, immunosuppressive, anti-nociceptive, antiepileptic, and antidepressant. In spite of containing such chemical defense compounds, the plant is attacked by several phytopathogens. A special group of microorganisms known as endophytes, which infect the internal tissues of plants without causing any immediate symptom of infection and live in mutualistic association with the hosts for at least a part of their life cycle, might be promising biocontrol agents providing ecologically and practically relevant solutions. For the first time, we have isolated a plethora of bacterial endophytes, both Gram-positive and -negative, from *C. sativa*. Bacteria are long known to communicate via quorum sensing auto-inducers (AHLs and peptides), sometimes causing specific damage and diseases. With this rationale, we are not only analyzing the profile of quorum quenching and/or enhancing responses of isolated endophytic bacteria to understand the interactions between Gram-positive and -negative isolates, but also evaluating their biocontrol potential in contributing to host plant defense. Consequently, this will help in elucidating plant-endophyte crosstalks and the necessary fitness-benefits provided by endophytes to the host plants.

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PPAP001

Differential expression of levansucrase genes in *Pseudomonas syringae*

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Pseudomonas syringae, an opportunistic plant pathogen, causes bacterial blight of soybean. It utilizes sucrose and produces exopolysaccharide levan by enzyme levansucrase. Levansucrase has three copies in this organism, two of which - *lscB* and *lscC*, are active while the third - *lscA* is inactive. Nucleotide sequence alignments of *lscB/C* variants in *P. syringae* showed a phage-associated promoter element (PAPE) upstream of the ORF which is absent in *lscA* [1]. Herein, we show that this upstream region is responsible the differential expression of *lscB/C* and *lscA*. For this we fused this PAPE (~450 bp upstream region and first 48 nucleotides of ORF) of *lscB* with *lscA* (called *lscBA*) and expressed this in levan negative mutant of *P. syringae* pv. glycinea PG4180. We also generated fusion of only the upstream region of lscB with lscA ORF (called *lscB'A*) and upstream region of *lscA* with *lscB* ORF (called *lscAB*). The *lscBA* and *lscB'A* showed levan formation while the *lscAB* did not indicating the *lscA* could be expressed in PG4180 under the *lscB* promoter. Native PAGE followed by MALDI-TOF anaylsis was performed to show that the levan formation in fusion proteins was indeed due to *lscA* and not due to *lscB/C*. qRT-PCR analysis showed that *lscBA* had an expression similar to *lscB* while *lscB'A* had lower expression. Both these results along with western blot analysis suggest that only the upstream sequence of *lscB* is sufficient for expression of levansucrase. We hypothesize that the PAPE might harbor binding sites for regulatory proteins possibly controlling sugar utilization in *P. syringae*. The upstream region of *lscA* is responsible for its cryptic expression since the enzyme itself is functional. We propose that *lscA* might be an ancestral levansucrase variant. The PAPE got inserted to its upstream region by phage-mediated genetic alterations through the course of evolution which led to an active levansucrase.

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PPAP002

AbcR1 sRNA regulates multiple ABC transporters in *Agrobacterium tumefaciens*

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Agrobacterium tumefaciens induces tumours, so-called crown galls, on plants upon transfer of a segment of its DNA (T-DNA) from its tumour inducing (Ti) plasmid to the plant nuclear genome (1). Wounded plants activate a complex defence programme in response to *A. tumefaciens*. They synthesize the non-proteinogenic amino acid gamma-aminobutyric acid (GABA), which stimulates degradation of the quorum sensing signal N-(3-oxooctanoyl) homoserine lactone. GABA is transported into *A. tumefaciens* via an ABC transporter dependent on the periplasmic binding protein Atu2422. We demonstrate that expression of *atu2422* is downregulated by the small RNA (sRNA) AbcR1 (2).
sRNAs are widespread regulators of gene expression in bacteria (3). The largest and most extensively studied set of sRNAs act through base pairing with targetRNAs, usually modulating the translation and stability of mRNAs (3). AbcR1 is the first described bacterial sRNA that controls uptake of a plant-generated signaling molecule. Proteome analyses with an AbcR1 deletion mutant revealed at least seven new targets. Four new periplasmatic substrate-binding proteins of ABC-transporters appeared to be silenced by this sRNA. Northern blot analyses confirmed the differential expression of the corresponding mRNAs in the AbcR1 deletion mutant. The molecular details of the sRNA-mRNA interactions will be presented.
Differential RNA sequencing (dRNA-seq) revealed more than 200 putative sRNAs distributed on all four *Agrobacterium tumefaciens* replicons, the circular chromosome, the linear chromosome, the At-plasmid and the Ti-plasmid (4). One sRNA from the Ti-plasmid was massively induced under virulence conditions.

Experiments to identify targets and to validate their involvement in virulence are under way.

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PPAP003

Molecular analysis of the role of LscR, a small regulatory protein involved in expression of levansucrase in different *Pseudomonas* species

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Pseudomonas syringae is a phytopathogenic γ-proteobacterium that induces a wide variety of leaf spots, speck, and blight diseases on various agronomically significant crops, as well as on an unknown number of wild plant species. Virulence of the bacterial blight pathogen of soybean, *Pseudomonas syringae* pv. *glycinea* PG4180, is favored by the temperature dependent production of levansucrase, an enzyme needed for production of the exopolymer levan and encoded by *lscB*. Expression of *lscB* in the heterologous host, *Pseudomonas putida* KT2440, required a PG4180-borne gene designated *lscR*. Our preliminary research focused on understanding the role of *lscR* in expression of levansucrase. Different plasmid constructs were transformed into *P. putida* KT2440 wild type and then grown on MG plates containing 5% Sucrose. Plates were incubated overnight at 28°C then transferred to 18°C and left for 10 days. Our results showed that KT2440 wild type and KT2440 transformants carrying either *lscR* or *lscB* are levan negative. However, when *P. putida* KT2440 carried both *lscR* and *lscB*, a positive levan phenotype was observed characterized by a white dome shaped colony. An interesting additional phenotype was observed when we compared the effects of two plasmid constructs: one carrying only a 1,350-bps region containing *lscR*, the other harboring a ~25-kb region contained in a cosmid and flanking *lscR*. The *P. putida* transformant carrying *lscB* and the 1,350-bps *lscR* region showed a levan-positive but 'swarming-out' phenotype. In contrast, the *P. putida* transformant carrying *lscB* and the cosmid showed a regular, evenly edged colony morphology without signs of swarming. While the 1,350-bps region contains only two small ORFs aside of *lscR*, the cosmid clone carries at least 23 different ORFs representing a prophage. Because of the exciting phenotypic differences between the transformants described above, it was hypothesized that the sole presence of *lscR* might lead to what we refer to as the "spreader phenotype" while presence of additional prophage-borne genes might negatively affect the *lscR*-mediated colony spreading.

PPAP004

Genome-wide transcriptome analysis of *Clavibacter michiganensis* subsp. *michiganensis* grown in xylem mimicking medium

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The interaction between *Clavibacter michiganensis* subsp. *michiganensis* with its host tomato is poorly understood, and only few virulence factors are known. While studying of the bacteria *in planta* is time-consuming and difficult, the analysis *in vitro* would facilitate research. Therefore, we established a xylem mimicking medium for *C. michiganensis* subsp. *michiganensis* based on an apoplast medium for *Xanthomonas campestris* pv. *vesicatoria*. Using this new medium, an increased *gfp*-fluorescence from a plasmid, which includes the *pat-1*-promoter, was measured. In microarray experiments, the expression of about 8 % of all *C. michiganensis* subsp. *michiganensis* genes was upregulated in xylem mimicking medium compared with minimal medium. The absence of sugars in xylem mimicking medium induced transcriptional changes of genes encoding putative sugar uptake systems. No transcriptional changes of hydrolytic enzyme genes were visible, what leads to the presumption that the medium reflects the condition in the beginning of an infection. After the addition of 20 μM of the wound substance acetosyringone, transcriptional changes were detected in microarray experiments compared with xylem mimicking medium alone. This medium serve as basic medium; with the addition of further compounds, more putative virulence factors could be detected and analyzed for their functions.

PPAP005

Role of the RNA chaperone Hfq in the plant pathogen *Agrobacterium tumefaciens*

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In the past years small noncoding RNAs (sRNAs) have received enormous attention as a new class of gene expression regulators. The largest and most extensively studied set of sRNAs act through base pairing with target RNAs, usually modulating the translation and stability of mRNAs [1].
The RNA chaperone Hfq directly influences the stability of RNA molecules and mediates the interaction between regulatory sRNAs and their mRNA targets [2]. Hfq affects bacterial physiology including growth, motility and resistance towards environmental stresses and plays an important role in microbe-host interactions as shown for a number of pathogenic and symbiotic bacteria [3].
Here, we demonstrate the significance of Hfq for fitness and virulence in the plant pathogen *Agrobacterium tumefaciens*. Deletion of *hfq* leads to reduced growth and motility, altered cell shape and attenuated virulence. By using differential RNA sequencing (dRNA-seq) technology we discovered more than 200 putative sRNAs in *A. tumefaciens* including the well characterized sRNA AbcR1 [4, 5]. Stability of AbcR1 and interaction with at least one of its mRNA targets (*atu2422*; periplasmic binding protein for GABA) are Hfq dependent [6]. Using Northern blot analyses we also identified mRNAs with increased stability in an *hfq* mutant.

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PPAP006

The Toxin-Antitoxin System RoaXY Regulates Synthesis of Aconitase B in the Phytopathogenic Bacterium *Xanthomonas campestris* pv. *vesicatoria*

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The plant pathogen *Xanthomonas campestris* pv. *vesicatoria (Xcv)* is an obligately aerobic, oxidase-negative γ-proteobacterium that causes bacterial spot disease on pepper and tomato plants. It grows in the intercellular space between plant cells where it must overcome iron- and oxygen-limitation, as well as cope with reactive oxygen species (ROS), which potentially form part of the host defence response. Moreover, *Xcv* must acquire enough carbon to proliferate in the plant apoplast. Little is known about the carbon sources used by *Xcv in planta*. Citrate is one carbon source present in apoplastic fluid that is potentially suitable as a growth substrate for *Xcv*. Aconitases (Acn) are iron-sulphur (Fe-S) proteins that catalyse the conversion of citrate to isocitrate in the TCA cycle. They are also responsive to both ROS and the cellular iron status and therefore might play an important role in plant pathogenesis. *Xcv* has three aconitases, two of which belong to the AcnA family and one that is an AcnB enzyme. Recent studies have shown that AcnB is important for growth of *Xcv in planta*[1]. The gene that encodes AcnB is co-transcribed with two small genes, termed *roaX* and *roaY*. The *roaXY-acnB* operon is highly conserved throughout xanthomonads. Synthesis of AcnB is induced upon entry into the stationary growth phase in *Xcv*. The *roaX* and *roaY* genes encode a putative DNA-binding transcriptional regulator and a RNase, respectively and deletion of *roaXY* leads to a 10-fold increase in AcnB levels, indicating that RoaX and RoaY regulate AcnB synthesis. Introduction of *roaX* and *roaY* into the *roaXY* mutant restored regulation of AcnB synthesis. Transcriptome analysis together with quantitative real-time PCR demonstrated that RoaXY regulate *acnB* gene expression and possibly also the stability of the *acnB* mRNA. Introduction of the gene encoding RoaX into the *roaXY* mutant of *Xcv* led to complete suppression of *acnB* expression.

We suggest that the RoaXY system regulates AcnB levels in response to a metabolic signal, possibly citrate levels, upon entry of *Xcv* into the stationary phase.

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PPAP007

Characterization of the domain of unknown function duf1521 conserved in a small set of pathogenic and symbiotic bacteria

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The conserved domain of unknown function DUF1521 was identified in a small set of proteins from α-, b-, γ- and δ-Proteobacteria, e.g. the coral pathogen *Vibrio coralliilyticus* ATCC-BAA450, the endophyte *Burkholderia phytofirmans* PsJN, *Brevundimonas diminuta* ATCC 11568, *Myxococcus xanthus* DK1622 and *Bradyrhizobium sp*. ORS285. The DUF1521 spans over approximately 170 amino acids and was first identified in proteins from the soybean symbiont *Bradyrhizobium japonicum* USDA110. Two homologous proteins, NopE1 and NopE2 each contain two of these domains. The NopE proteins were shown to be expressed during symbiosis and are translocated by a type III-secretion system into the cytosol of host cells (Wenzel et al. 2010). NopE1 and NopE2 show self-cleavage activity at a conserved site inside the DUF1521. The cleavage is inducible by calcium in vitro and can be inhibited by EDTA. The function of this conserved domain in *Bradyrhizobium*-plant interaction is currently investigated. Interestingly, the presence of the DUF1521-containing proteins is detrimental for symbiosis of *Bradyrhizobium japonicum* on some and beneficial on other host plants. A *nopE1*/*nopE2* double mutant shows high nodulation efficiency on *Vigna radiata* cv. KPS2, whereas the wild type nodulates this plant very poorly. Although NopE1 contains two DUF1521 domains, only one self-cleaving domain is sufficient to complement the mutant phenotype on *V. radiata* KPS2.
DUF1521 domains from *Sphingomonas* sp. SKA58, *V. coralliilyticus* and *B. phytofirmans* were analyzed. These domains contain the conserved cleavage site motif and show self-cleavage activity in vitro. The DUF1521 domain of the *Vibrio coralliilyticus* protein Vic_001052 was analyzed in further detail. Experimental data suggest a functional conservation of the DUF1521 domains.

References:
Wenzel, M., Friedrich, L., Göttfert, M., Zehner, S. 2010**.** The type III-secreted protein NopE1 affects symbiosis and exhibits a calcium-dependent autocleavage activity. Mol. Plant-Microbe Interact. 23, 124-129.

PPAP008

First Report of pathogenic fungi infecting Marama bean in Namibia

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Tylosema esculentum (Marama bean) is nutritous legume that is endemicin the Southern Kalahari (Namibia, Botswana and South Africa) agroecology. It is well adapted to the dry, deep sandy soils of the Kalahari and it is drought-avoiding. It promises to be the futurer legume crop for dry lands, especially those heavily influenced by climate change. The seeds are rich in essential oils and protein. However, very little research has been done on this plant. Due to its agricultural potential, domestication research has been initiated at the University of Namibia. The aim of the study was to survey for pathogens affecting growth and production of marama bean and to determine the identity of these fungal pathogens. Diseased pods presenting necrotic spots with dense sporulation in the centre were collected. They were subjected to fungal isolation using Potato Dextrose Agar (PDA) at room temperature under fluorescent light for five days. Two fungi with different cultural conidial morphology were isolated. Single spores from the two cultures were separately inoculated on PDA to obtain a pure culture. Fungal genomic DNA was extracted from fresh mycelium using the CTAB method and then used as a template in ITS PCR amplification. Based on the sequence analysis of the 5.8S ribosomal DNA and the internal transcribed spacer fragment (ITS), the two isolates associated with necrotic Marama pods were identified as *Alternaria tenuissima* and a *Phoma* species due to the high sequence homology which was 99% in both cases. This is the first report describing the presence of these two fungi on co-infecting marama bean. This has serious production implications to be considered as marama bean domestication gets underway.

PPAP009

A *Sporisorium reilianum* variety isolated from maize induces several plant responses in sorghum.

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Smuts are biotrophic phytopathogenic fungi that present a very narrow host range. *Sporisorium reilianum* is a member of this group and exists in two host-adapted varieties that cause head smut on sorghum (*S. reilianum f. sp. reilianum*) or maize (*S. reilianum f. sp. zeae*). To investigate the differences leading to host specificity of *S. reilianum*, we microscopically followed each variety during colonization of sorghum. Both varieties were able to penetrate and multiply in sorghum leaves. Hyphae of *S. reilianum f. sp. reilianum* preferentially colonized bundle sheath cells, entered the vascular bundles and reached the apical meristem. In contrast, hyphae of *S. reilianum f. sp. zeae* did not show a preference for any plant tissue and were never found in apical meristems. While fungal DNA of *S. reilianum f. sp. reilianum* was prominent in inoculated leaves, nodes and apical inflorescences, relative DNA quantity of *S. reilianum f. sp. zeae* decreased from the inoculated leaves to the other parts. Likewise, *S. reilianum f. sp. zeae* induced a succession of defense responses in sorghum, which were observed by microscopy of stained samples and quantitative real time PCR of marker genes. Penetrating and proliferating hyphae of *S. reilianum f. sp. zeae* induced a local H_2O_2 response at 1 day after inoculation (dai). At 2 dai, proliferating hyphae met with fortified cell walls containing callose, and at 3 dai a strong phytoalexin accumulation was observed. Our data suggests that host specificity of *S. reilianum* is the result of a multilayered adaptation to either suppress or evade the different plant defense responses.

PPAP010

Successful colonization of maize by *Sporisorium reilianum* is dependent on effector proteins

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Sporisorium reilianum infects maize at seedling stage but symptoms are visible only at flowering time. Symptoms include the formation of spores and phyllody in the inflorescence. Genome comparison between *Sporisorium reilianum* and the closely related fungus *Ustilago maydis* that causes gall formation on maize leaves and flowers discovered the presence of genomic regions of weakly conserved genes mainly encoding secreted proteins. Deletion of the largest divergence region of about 30 genes in *S. reilianum* dramatically reduced virulence, and led to wilting of inoculated leaves. By subdeletion analysis we identified a region encoding three related secreted effector proteins responsible for the early leaf wilting phenotype. Two effector candidates contribute to the virulence and the suppression of early leaf wilting. Both genes are up-regulated during biotrophic growth of *S. reilianum* as determined by qRT-PCR analysis. Yeast two-hybrid analysis revealed potential interaction partners that are involved in hormone production and plant disease resistance. This suggests a potential mechanism of effector function by modulating plant hormone levels, or suppressing plant defense.

PPAP011

Molecular investigation of genes involved in sugar metabolism in plant pathogen *Pseudomonas syringae*

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Pseudomonas syringae pv. glycinea PG4180, the causative agent of bacterial blight of soy-bean plants, use the extra-cellular enzyme, levansucrase, which is encoded by two genes *lscB* and *lscC*, to metabolize plant borne sucrose into glucose and fructosyl residues. These fructosyl residues are polymerized to form an expolysaccharide called levan. Although *P. syringae* M6 mutant is deficient in expression of both *lsc* genes and does not produce any levan, it interestingly can grow in a liquid minimal medium containing sucrose as its sole carbon source indistinguishably from the wild type PG4180. Consequently, it was hypothesized 'that a second gene product(s) could be present which enables the M6 mutant to grow on sucrose-containing medium.' To answer this question, literature search was performed to find other enzymes potentially involved in the sucrose metabolism. The search led us to the identification of an intracellular enzyme, sucrose-6-phosphate hydrolase (ScrB), which cleaves sucrose into glucose/glucose-6-phosphate

and fructose. Consequently, it is planned to mutagenize the *scrB* gene which encodes for this enzyme by generation of a mutagenic construct using PCR cloning and subsequent homologous recombination into the genomes of the PG4180 wild type and mutant M6. The effect of this mutation on the sucrose utilization by wild type and mutant M6 will be investigated using growth experiments with different carbon sources. Additionally, the expression of *scrB* and *lscB/C* will be analysed in respective mutant backgrounds using qRT-PCR.

PPAP012

How specific is specific? Are endophytic bacteria living inside plant calli naturally selected by the host?

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Endophytic bacteria are found in almost all plant organs, like roots, stems, leaves, seeds or fruits. The population density of endophytes is highly variable and seems to depend mainly on the bacterial species or host genotype as well as on the developmental stage of the host and environmental conditions (Hartmann *et al*. 2009). In the DSMZ collection of plant cell cultures about 700 undifferentiated plant cell lines with a main focus on secondary metabolite producing cultures are maintained as calli since more than 20 years. They are widely used for fundamental and applied research. In a new research project (Cost Action FA1103) calli previously selected for possible contamination were investigated for endophytes using culturing methods as well as DNA- and RNA-based cloning approaches. The goal of this study is to identify hidden endophytes and to uncover potential interactions between a callus and its microbial community. Until now no information about effects of endophytes on plant calli is available and potential physiological and molecular interactions have not been investigated. Interestingly, bacterial species of the genus *Paenibacillus* were isolated from three calli of different plant families, i.e. *Ludwigia octavalvis* (Jacq.) P. H. Raven (Onagraceae), *Melanoselinum decipiens* (Schrad *et* H. L. Wendl.) Hoffm. (Umbelliferae) and *Stephania wightii* Dunn (Menispermaceae) and detected as well in clone libraries. Another plant species, *Catharanthus roseus* (L.) G. Don (Apocynaceae), shows the presence of a *Mycobacterium* species inside the callus. Normally specific endophytes are recruited by plants out of a large pool of soil or rhizospheric species (Hartmann *et al*. 2009). In the present study on calli it will be tested, if detected bacteria are "true endophytes" with capacity to infect non-infected calli of the same species bringing benefits to the host, or are just "contaminants". In a first screening approach, isolated bacteria from calli were investigated in serial dilution tests for their biological activity. If these bacteria originate from a rhizospheric bacterial community they may possibly exudate antimicrobial compounds. Preliminary results show that extracts of *Paenibacillus* species show weak activity against Gram-positive bacteria and fungi.

PPAP013

Grassland species diversity increases abundance and disease suppressive ability of *Pseudomonas fluorescens*

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Soil bacteria such as pseudomonads function as biocontrol agents, due to their inhibiting potential against plant pathogens. In order to foster bacteria-mediated plant protection, factors affecting the dynamics of plant growth promoting rhizobacteria (PGPR) need to be understood. Plants act as drivers of rhizosphere bacterial communities. Our recent results showed that in temperate grasslands increasing plant diversity enhances the abundance of plant pathogen antagonistic pseudomonads. However, the effect of plant diversity and identity on antagonistic bacterial activity is still unknown. We investigated the effect of plant diversity on the expression of antifungal genes by using established GFP reporter fusions of the model biocontrol strain *Pseudomonas fluorescens* CHA0 in the rhizosphere of plants grown alone or in mixed cultures. According to our recent results, antifungal gene activity increased with plant species diversity and in presence of grasses, whereas legumes negatively affected gene activity. The results yet again underline the importance of biodiversity for ecosystem functioning. Plant polyculture may foster plant-protective bacterial communities and the use of certain plant combinations may help establishing environmentally friendly control of plant diseases.

PPAP014

Molecular characterization of Pseudomonas savastanoi isolates from Mandevilla sanderi

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In 2008 breeders of the ornamental plant *Mandevilla sanderi* observed for the first time large necrotic lesions with chlorotic rings on leaves and tumor formation on stems. The potential causal agents isolated from the lesions of leaves of diseased plant material were identified initially by metabolic profiling (BIOLOG) as *Pseudomonas savastanoi* pv. glycinea or pv. nerii. Several pathovars of *P. savastanoi* infect woody plants, e.g., *P. savastanoi* pv. savastanoi is known as an important pathogen of olive trees (*Olea europaea*) in the Mediterranean area.
The present study was carried out to perform a molecular characterization of *P. savastanoi* isolates from *M. sanderi* and to compare them with isolates originating from olive trees, oleander, jasmine and privet. Furthermore, we aimed to use this information as the basis for the development of a sensitive and specific method for detection and differentiation of the pathogen from total community DNA.
The BOX fingerprints were similar for *P. savastanoi* isolates from different host plants. All sequences were 100% identical. Phylogenetic analysis of partial nucleotide sequences (1,413 bp) of the 16S rRNA gene showed that the isolates from *M. sanderi* clustered together with those of *P. savastanoi* pv. nerii (ITM313) and *P. savastanoi* pv. savastanoi (NCPPB 3335). All *P. savastanoi* isolates contained plasmids. Plasmid restriction patterns and sequencing of plasmid-located pathogenicity determinants revealed that all *Mandevilla* isolates contained similar plasmids distinct from those of isolates from other host plants. A PCR-based system for detection of *P. savastanoi* in isolates and total community DNA from *M. sanderi* was developed based on the *repA* gene as amplicons of the right size (1,100 bp) were obtained only from *P. savastanoi* isolates from *Mandevilla* isolates. The system was successfully used to detect the pathogen in total community DNA extracted from leaf and tumor material of plants inoculated with strain Ph4 by means of a PCR amplification and hybridization with a PCR generated digoxigenin labeled *repA* probe. The hybridization method is an important means to increase not only the sensitivity but also the specificity of detection. The application of the detection system can be also used to identify isolates as well as to detect the pathogen in total community DNA directly extracted from plant material or soil.

PPAP015

The localisation of the type III-secreted protein NopE1 and its role in symbiosis of *Bradyrhizobium japonicum* with legumes

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The nitrogen-fixing symbiotic bacterium *Bradyrhizobium japonicum* is a slow growing soil bacterium and secretes various proteins (Nops, nodulation outer proteins) via a type III secretion system (T3SS) (1,2). These secreted effectors affect the symbiotic potential of *B. japonicum* with their leguminous host plants. The type III secreted effector protein NopE1 was shown to be expressed during symbiosis with *Macroptilium atropurpureum* (3). Biochemical analysis of the purified protein shows that in the presence of calcium ions NopE1 is cleaved into three characteristic fragments. To get a general idea of the localization of NopE1 and its role in symbiosis nodule extracts were analyzed. For this, different antibodies were used. Specific polyclonal antibody were raised against recombinant NopE1. Furthermore, NopE1 of *B. japonicum* was tagged with a 2xFlag. Both experimental approaches show that NopE1 is localized in the cytosol of infected nodule cells. The sub-fractionation of nodule extracts (in symbiosome-, bacteroid- and cytosolic fraction) reveals that NopE1 is likely to be cleaved after secretion into the plant cytosol. Nodulation assays indicate that the presence of functional (cleavable) NopE1 has no influence on symbiosis with *Glycine max*. and *M. atropurpureum*. Interestingly, in the interaction with *Vigna radiata* cv. KPS2 the cleavage of NopE1 is an essential aspect for the nodulation capability of *B. japonicum*.

Only the absence of NopE1 or presence of only non-cleavable (non-functional) NopE1 leads to functional nodules (4).

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PPAP016

The effect of soil type and potato plant spheres on the abundance and diversity of bacterial antagonists of *Ralstonia solanacearum*

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Ralstonia solanacearum is an epidemic quarantine pathogen responsible for brown rot disease in potato and can infect a very broad range of plant hosts under different climatic conditions. This pathogen can survive in the soil for years and can spread very fast via water streams. Biocontrol agents can reduce *R. solanacearum* infection but the performance can vary due to the competition with indigenous soil microbial communities and adaptation to harsh environmental conditions. The aim of this study is to assess the effect of soil type on the abundance and diversity of antagonistic bacteria and total microbial communities in soil and potato plants. The microbial communities were compared by PCR-DGGE fingerprints of 16S rRNA genes amplified from total community DNA. Two thousand bacterial isolates obtained from different plant spheres grown in three different soils (Diluvial sand, Alluvial loam and Loess loam) were screened for in vitro antagonistic activity towards *R. solanacearum* and 400 isolates with antagonistic activity were further characterized. The total microbial communities DGGE profile showed distinct microbial community structures associated with each plant sphere, and within each plant sphere the microbial community composition was mainly shaped by the soil type, nevertheless the effect of soil type decreased in the endophytic compartments, suggesting that this endophytic compartment harbored a unique microbial community protected from surrounding environment. Currently, we investigate whether endophytic antagonistic bacteria with different antagonistic mechanisms can effectively eradicate and prevent *R. solanacearum* infection.

PPAP017

Soil type and plant species affect rhizosphere microbial community composition and biocontrol of *Rhizoctonia solani*

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The soil borne pathogen *Rhizoctonia solani* is difficult to control with fungicides. The use of antagonistically active bacteria could be an environmental friendly alternative. The strain *Pseudomonas jessenii* RU47 showed already remarkable biocontrol effects under laboratory and greenhouse conditions. However, the biocontrol activity of inoculated bacteria under field conditions is often inconsistent. The reason for this variability is until now unknown. An important factor influencing the crop yield, plant health and disease symptoms is assumed to be the soil type. Soils differ in chemical and physical conditions which may lead to different microbial communities. To understand the complex interactions between the soil type, plant and microbial community a field experiment with a plot system containing three soil types and two plant species was performed. The dry weight and disease severity of lettuce and potato was analyzed as well as the rhizosphere colonization of the biocontrol strain. The soil type did not affect plant dry weight, but there were differences in the establishment of the pathogen. Surprisingly, the colonization density of the biocontrol strain was independent from the soil type, as determined for lettuce. PCR-DGGE analysis showed that the rhizosphere microbial community composition of lettuce and potato in the three soil types differed significantly. The biocontrol strain had a negligible effect on the indigenous microbial community. Amplicon pyrosequencing of 16S rRNA genes from bulk soil and rhizosphere samples from lettuce revealed that *Proteobacteria, Actinobacteria, Firmicutes, Acidobacteria* and *Bacteroidetes* were the dominant phyla in all three soil types. The fast growing *Proteobacteria* were enriched in the rhizosphere compared to bulk soil whereas the relative abundance of the other dominant groups decreased. The *Firmicutes* were enriched in one of the loamy soils, but decreased in the other two soils. A detailed analysis of the OTU report showed that many OTUs were enriched in the rhizosphere of lettuce independent of the soil type but also a noticeable number of OTUs are not influenced by the rhizosphere environment. Also there are significant differences between the soil types especially the two loamy soils seem to enrich other OTUs than the sandy soil.

PPAP018

Inhibition of *Verticillium dahliae* by *Streptomyces lividans*

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Verticillium wilt causes immense crop losses. We discovered that the Gram-positive, spore-forming soil bacterium *Streptomyces lividans* reduces the vegetative growth, the formation of conida and microsclerotia of *Verticillium dahliae* growing alone or on roots of the model plant *Arabidopsis thaliana* (1). During this interaction, *S. lividans* produces two members of the prodiginine family, undecylprodigiosin and streptorubin B. The addition of purified undecylprodigiosin to growing *V. dahliae* hyphae strongly reduced microsclerotia formation. Based on these novel findings, we deduce that the prodiginine investigated leads to multiple cellular effects, which ultimately impair specific pathways for signal transduction and apoptosis of the fungal plant pathogen in the absence or presence of *Arabidopsis thaliana* (2). Ongoing studies reveal that additional compounds play a role.

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PMEV001

Unexpected microbial drivers of N_2O fluxes in arctic permafrost-affected peatlands

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Permafrost-affected peatlands were recently identified as sources of the greenhouse gas N_2O. Unvegetated tundra peat circles (PC) are nitrate-rich 'hot spots' of N_2O emissions, while adjacent vegetated tundra areas (UT), and moderately vegetated palsa peats (PP) emit only minor amounts of N_2O. Soil pH approximated 4 for all sites. N_2O emission potentials of unsupplemented anoxic peat soil slurries and apparent maximal nitrate-dependent reaction velocities were highest for PC, intermediate for PP, and lowest for UT. Stimulation of N_2O production by nitrate-, nitrite-, and/or acetylene suggested denitrification as major source of N_2O in all soils. $N_2O/(N_2+N_2O)$ ratios were 30, 55, and 100% for PC, PP, and UT, respectively, when 10 μM of nitrate were supplied. Such dissimilar physiological responses suggested dissimilar denitrifier communities in the soils. Barcoded amplicon pyrosequencing of *narG*, *nirK*/*nirS*, and *nosZ* (encoding nitrate, nitrite, and N_2O reductases, respectively) yielded 40,000 sequences, revealed diverse denitrifiers including hitherto unknown species, and likewise suggested that denitrifier diversity differed between the soils. Sequences affiliated with *Actino-, Alpha-* and *Betaproteobacteria* as well as environmental sequences. Quantitative PCR of *narG*, *nirK*, *nirS*, and *nosZ* indicated that detected *nirS* outnumbered *nirK* in all soils. Detected *narG* and *nirK* were most abundant in PC. The collective data suggest that (i) *Proteobacterial nirS*-type rather than *nirK*-type denitrifiers are abundant in acidic permafrost soils, and (ii) the contrasting N_2O emission patterns are associated with contrasting denitrifier communities.

PMEV002

Microbial communities associated with buried carbon in cryoturbated soils of the Siberian Arctic and their response to a warming climate

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Arctic permafrost regions contain an estimated 1672 Pg of soil organic carbon (SOC), an amount twice as much carbon as is contained in today's global atmospheric carbon pool (Tarnocai et al. BGC 2009). More than one third of this SOC is stored in cryoturbated soils, its degradation being strongly retarded and thereby removed from the current carbon cycle (Kaiser et al. JGR 2007). Key microbial regulators in retarded SOC degradation might be (1) a reduction in microbial community size accompanied by lower degradation activities, and/or (2) a shift in community composition. To test these hypotheses, soil samples were collected in the Kolyma area and on the Taymyr Peninsula (Eastern and Central Siberia) and a comprehensive set of geochemical data, microbial community profiles and enzyme activities was generated in the framework of the international research project CryoCARB (www.cryocarb.net).
Cryoturbated layers harboured an unexpectedly high amount of bacteria, archaea and fungi. Bacterial and archaeal communities in the cryoturbated soils were clearly distinguishable from the organic topsoil, but similar to the subsoil communities. The relative abundance of potential indicator taxa and functional groups (Firmicutes, Desulfuromonadales within the Deltaproteobacteria, methanogens) increased from the topsoil to the subsoil indicating a change in redox conditions and a shift from aerobic to anaerobic microorganisms with depth. In contrast, the relative contribution of fungi was significantly lower in cryoturbated layers than in unturbated soils most likely due to the reduction of mycorrhizal basidimycota. As they play an important role as strong decomposers, reductions in the size of such a major group of soil organisms could reduce the quantity of enzymes involved in SOC degradation and subsequently reduce the rate of decomposition. Furthermore, laboratory incubation experiments indicated a strong temperature response of bacterial, archaeal and fungal communities in cryoturbated layers reflected by an increase in SSU gene abundances, enzymatic activities and methane accumulation in anoxic incubations. Our results support the hypothesis that community structure is a key factor in SOC stabilisation in cryoturbated soils and will significantly determine their response to a warming climate.

PMEV003

Benthic bacterial communities in a changing Arctic Ocean - a case study on the Laptev Sea margin (Arctic Ocean)

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Benthic communities of the Arctic seafloor depend on the sedimentation of particulate matter from the sea ice and the water column, which is determined by the vertical export flux from the ice and the euphotic zone, and by lateral supply from shelf areas. One of the central questions as to the consequences of the shrinking sea ice cover is to what extent primary production and subsequent export of matter to the seafloor will be affected, and how this will influence the structure and functioning of benthic communities in the Arctic. Previous studies of the Laptev Sea continental slope revealed strong relationships between phytodetritus availability and benthic bacterial community structure and activity (Boetius & Damm 1998, Deep-Sea Res. I 45:239; Bienhold et al. 2012, ISME J 6:724). The samples analyzed in these studies originate from the 1990s when the Laptev Sea was largely ice-covered throughout the year. They offer a unique ecological baseline against which ecosystem shifts can be assessed. In the past decade, a rapid decline in sea ice cover has occurred, leaving most of the investigated area ice-free during the Arctic summer. The Polarstern expedition IceArc (ARK-XXVII/3) returned to the area in September 2012 to resample the same sites between 60 and 3400 m water depth. The results suggest that environmental changes in the past two decades may have led to a substantial increase in phytodetritus input to the seafloor. Based on our previous studies, we hypothesize that changes in phytodetritus availability at the seafloor will have likely caused shifts in bacterial community structure and activity. We therefore compare bacterial biomass, community structure, extracellular enzymatic activities and microbial oxygen uptake between the years 1993 and 2012, and evaluate them in conjunction with environmental parameters (e.g. chlorophyll pigments, ice cover). This comparative study carried out in the framework of the ERC project ABYSS provides insights into bacterial community dynamics in a region dramatically influenced by global change over a time frame of two decades and will help to assess the fate of Arctic benthic ecosystems under future climate scenarios.

PMEV004

The El'gygytgyn Crater Lake - an example for the deep-biosphere in up to 3.6 million year old lake sediments

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The El'gygytgyn Crater Lake, Chukotka, Far East Russian Arctic, was formed by a meteorite impact 3.6 million years ago [1] and is characterized by a continuous accumulation of lake sediments due to lack of glaciations in this region. The chronological layers contain information about how the Arctic has responded to the various glacial/interglacial periods of the past millennia. Moreover these sediment layers preserve traces of previous live, e.g. in form of pollen, diatoms, or biomarker such as lipids or amino acids.
An initial investigation of the microbial composition in up to 470 ka old sediment deposits revealed a diverse archaeal community regarding the chronosequence with a strong correlation between the amount of organic carbon and the microbial abundance and diversity. In these parts of the sediments the microorganisms showed an indirect response to glacial/interglacial periods, which can be explained by the improved nutrient supply in course of related algal bloom [2].
Further analyses of the entire sediments down to 317 m with an age of up to 3.6 million years should provide detailed knowledge about the pioneering microorganisms of the El'gygytgyn Crater Lake after the impact. In addition changes of the composition and abundance of the microbial community along the chronosequence is to be examined. Another goal is to verify the existence of metabolically active microorganisms in this ancient sediment material. Therefore a propidium monoazide (PMA) protocol optimized for sediment material will be performed. PMA is a DNA intercalating dye to distinguish between free DNA and intact cells, which is well developed for bacterial pure cultures [3].
A broad set of analytical methods (including ribosomal DNA gene based approach, cultivation and fluorescence in situ hybridization), biogeochemical techniques and phylogenetical analyses on the El'gygytgyn sediment material should improve our understanding of the development of microbial life and provide information about the limitations of microbial activity.

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PMEP001

Isolation and characterisation of heterotrophic micro-organisms from glacier forefields of the Larsemann Hills, East Antarctica

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Extreme environments like glacier forefields in Antarctic areas are microbial dominated ecosystems. Several studies revealed the presence of divers and specialised microbial communities. However, the function of microorganisms in the habitat often remains unclear because identified

species are unknown due to the lack of isolates. In the background of environmental studies in two glacier forefields of the Larsemann Hills in East Antarctica 148 heterotrophic bacteria were isolated and identified. Eight strains were characterised in detail and strain PB1^T and PB4^T could be described as novel species *Candidatus Herbaspirillum psychrotolerans* [1] and *Candidatus Chryseobacterium frigidisoli* [2], respectively. The gram-negative strain PB1^T is the first psychrotolerant (T_{range} = -5 to 25°C) isolate in the genus *Herbaspirillum* with optimum temperature for growth at 20°C. Strain PB4^T is a gram-negative and psychrotolerant (T_{range} = 0-25°C) representative in the family *Flavobacteriaceae*, which can use a wide range of substrates as sole carbon source and produces acids from various carbohydrates. The isolation and characterisation of microorganisms in extreme environments is important to understand their role in primary succession and biogeochemical cycling.
Furthermore cold adapted species have a unique potential in biotechnology.

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PMEP002
Methanotrophy in aquatic ecosystems of the Lena Delta, Northeast Siberia

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Great amounts of methane, a very potent greenhouse gas, are produced via anaerobic decomposition of organic carbon in permafrost-affected soils and aquatic sediments of the Lena Delta area, Northeast Siberia. With predicted global climate change permafrost thaws and more methane will escape to the atmosphere through soils and water bodies. Methanotrophic bacteria can act as counterpart of these processes and as important sink of methane in these ecosystems.
The aim of this research was to investigate the distribution of methane and to determine methane oxidation rates (MOX) in lakes and streams of the Lena Delta and in the Lena River itself as well as to analyze the physico-chemical parameters which can affect methanotrophy in changing environments (suspended particulate matter, light, salinity).
The main investigations were conducted on the Samoylov Island, located in the central-south part of the Lena-Delta and along the Bykovskaya Protoka, one of the largest Lena River channels. Lakes and streams along the shore of the Samoylov Island and nearby Lena River were the main sampling sites. Methane concentrations were determined using gas chromatography, methane oxidation rates were counted following radiotracer (tritiated methane) technique using liquid scintillation counter.
Investigation revealed that methane concentrations in lakes and streams varied from relatively low (200 nM) to extremely high (20 μM) concentrations and were decreasing in the river near the shore and in the middle. Turbidity (suspended particulate matter) and salinity but not light influenced the MOX.
Thus some lakes and streams of the Lena Delta contain high amounts of methane which in combination with high methane oxidation rates, reveals that methanotrophy plays an important role in carbon cycle of this aquatic ecosystem.

PMEP003
Genomics of *glaciecola sp.*, a predo-minant bacterium in antarctic winter sea-ice

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About 20 Million km^2 of sea ice covers the Southern Ocean in winter which retreats to about 6 Million km^2 in summer. In spite of the harsh temperature and salinity conditions a so-called sea-ice microbial community thrives within the liquid brine system. Sea-ice organisms are very closely adapted to their habitat. Although in recent years several genomic projects started with bacteria from polar sea ice the genomic basis of their adaptation strategies is still unknown. Hence, further complete genome sequences of other members of the sea-ice communities are required to improve our understanding. For a complete genome sequencing the bacterium *Glaciecola sp.* was chosen which constituted more than 25% of our isolates from winter pack ice of the Weddell Sea and was also regularly isolated from Arctic summer sea ice. The nearest relatives of this group on the 16S rRNA gene basis are the type strains *G. psychrophila* and *G. mesophila*. The complete genome sequence of *Glaciecola* sp. 9081 comprises one circular chromosome with 4.99 Mega base pairs. A total of 5800 open reading frames was predicted. Based on annotation of proteins by sequence similarity we found genes for the production and transport of osmolytes that may balance the osmotic pressure of the cell when sea ice freezes. Further, genes involved in the production of extracellular polysaccharides were detected that may play a role in cold adaptation by lowering the freezing point in the vicinity of the cell. In ongoing studies we are trying to unravel further molecular concepts of cold adaptation.

PMEP004
Exploration of microbial biodiversity in polar glacial ice

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The polar ice caps play a key part in providing an understanding of climate variability over the last eight glacial cycles and may give information about paleoenvironmental features and changes of microbial diversity in the past. Microbiological studies on polar ice cores are rare and focused so far on silty or accreted ice. Aim of our studies is to detect, characterize, and compare the prokaryotic diversity in different Arctic and Antarctic ice cores at different core depths in order to learn more about relations of past and recent communities and about alterations of ancient communities in relation to climatic changes. Critical points in ice core analytics are the strong contaminations of the outside of ice cores as well as the limited availability of sample material. Hence, preparation/decontamination protocols were tested and adapted with inoculated and artificially contaminated ice cores. Our work on real older glacial ice started with material from the uppermost 200m of the ice shield of Dronning Maud Land. We detected some pollen grains and a low abundance of bacteria cells by Sybr Green staining and solid phase cytometry in ice core meltwater. From 3 other ice core samples from about 130 m depth, approximately 2150 years old, DNA could be extracted and amplified. DGGE-analyses of amplified DNA revealed a low bacterial diversity. Most of the DGGE-bands occurring in the inner ice core were also present in blanks and controls. One strong band however, was detected only in the inner part of two of the three ice cores analyzed. This band could be affiliated to the alpha-proteobacteria with a high similarity to *Bradyrhizobium japonicum*. Clone libraries and metagenomic studies supported the dominance of this bacteria type that might jointly be responsible for N_2O abnormalities in glacial ice cores.

PMEP005
Organic carbon transformations in Arctic peatlands: key functions and microorganisms

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Question: A substantial part of the Earths´ soil organic carbon (SOC) is stored in permafrost-affected peatlands, which represent large potential sources for increased emissions of the greenhouse gases CH_4 and CO_2 in a warming climate. The microbial communities responsible for the mineralization of SOC to CO_2 and CH_4 in these soils and their response to a warming climate are, however, poorly understood.
Methods: Here, we applied a combined metagenomic and metatranscriptomic approach on two high-Arctic peat soils from Ny-Ålesund, Svalbard. Furthermore, the influence of temperature was investigated in anoxic microcosms.
Results: The gene pool for plant polymer degrading enzymes was not different to the ones found in metagenomes of soils from other climatic zones. The majority of these genes were assigned to three bacterial phyla, Actinobacteria, Verrucomicrobia and Bacteroidetes. Genes and transcripts of anaerobic metabolic pathways and the fraction of methanogenic archaea increased with depth, suggesting a gradual transition to anaerobic lifestyles. Methanotrophic bacteria closely related to *Methylobacter tundripaludum* comprised more than 2% of the peat microbiotas. The influence of temperature on anaerobic peat decomposition was investigated in

microcosms experiments. CH_4 accumulation and hydrolytic enzyme assays showed a non-linear response to temperature with high activities at low temperatures. This was reflected also in shifts of the microbiota structure and function, as assessed by metatranscriptomics.
Conclusions: This study helps to shed light on the microbial key populations in arctic peat soils and on their possible response to higher temperatures. We suggest that SOC mineralization will likely increase in a future warming climate. The magnitude of future CH_4 emissions however, will not only depend on the response of anaerobic processes, but also on the yet unknown response of methanotrophic bacteria.

PMEP006
Microbial communities of the methane cycle associated with mosses of a sub-arctic peatland

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Northern peatlands are one of the major natural sources of the greenhouse gas methane. Bryophytes such as Sphagnum and the so called brown mosses are major constituents of the plant community of those peatlands and can be important habitats for microbial symbionts. Methane oxidizing bacteria associated with Sphagnum were shown to reduce methane emission from peatlands, and especially submerged habits of Sphagnum show high methane oxidation activities (KIP et al., 2010). Most studies were concerned with Sphagnum, but little is known about the microbial communities associated with brown mosses despite their considerable contribution to reduce methane emissions e.g. from Arctic polygonal peatlands (LIEBNER et al., 2011). We quantified the microbial communities associated with dominant mosses of a sub-Arctic, acidic peatland applying quantitative (real time) PCR. We examined endophytic bacterial and archaeal communities as well as methanogenic and methanotrophic symbionts of a Drepanocladus species, Sphagnum lindbergii and Sp.riparium, which thereby appeared both as emerged and submerged grown plant. Our results showed clear differences in the abundance of the moss symbionts. While the brown moss Drepanocladus harbored large communities of endophytic symbionts (e.g. up to 2 x 1010 bacteria per gram dry moss), bacterial cell numbers of the emerged Sp.riparium and Sp. lindbergii were below 108 per gram dry moss. Pronounced differences were also found between the submerged and the emerged grown Sp.riparium with clearly higher microbial cell numbers in the submerged moss. Our results suggest that within the same peatland moss taxon and water level influence the size of moss-associated microbial communities of the methane cycle and with this presumably also important processes like methane oxidation and methane formations. N. Kip ,J.F. van Winden, H.J.M. Op den Camp, Nature Geoscience 3 (2010), pp. 617 - 621 "Global prevalence of methane oxidation by symbiotic bacteria in peat-moss ecosystems" T. Larmola, E.-S. Tuittila, H. Fritze Ecology 91/8 (2010), pp. 2356-2365 "The role of Sphagnum mosses in the methane cycling of a boreal mire" S. Liebner, J. Zeyer, C. Knoblauch, Journal of Ecology (2011) "Methane oxidation associated with submerged brown mosses reduces methane emissions from Siberian polygonal tundra"

PMEP007
New estimates of primary productivity and its limiting factors in the Central Arctic

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The Arctic Ocean is changing dramatically due to the rapid sea ice decrease. The reduction in ice thickness and cover leads to an increase in the amount of light penetrating through the ice. Thus changes in primary production are expected. Nevertheless, our knowledge about how other limiting factors such as nutrients might affect budgets and ratios of sea ice algae and phytoplankton productivity is still limited, especially in the central basins.
This study presents recent results from the Central Arctic Ocean collected during late summer and early autumn 2011 and 2012. Primary productivity was measured using the 14C method in the water column, the sea ice and in the melt ponds through a wide variety of ice types and water masses. Additionally PI curves and nutrient enrichment experiments were performed at selected stations in order to infer primary productivity´s limiting factors at the end of the season.
Aggregates of sea ice algae found in melt ponds and directly under the ice showed hardly any photoinhibition and presented the highest productivity on small scales . Nevertheless their contribution to the total primary production remains difficult to assess due to their patchiness. Regarding the different ice types, multi-year ice contained more algal biomass than first year ice at the end of the summer, but due to its much larger spatial extent and higher light transmission throughout the season, first year ice is likely to provide a higher proportion of overall sea ice algal productivity in a changing Arctic. The ice-covered water column had in general very low NPP rates probably due to both light and nutrient limitation, and the depth of the mixed layer in the Eurasian basins was limited to 15 m in 2012. Therefore in a summerly ice free Arctic Ocean, the proportional contribution of sea ice and sub ice algae compared to phytoplankton in highly stratified, nitrate limited surface waters is still difficult to predict, and will depend on nutrient transport and mixing processes.

PRMV001
Metabolic networks and carbon and nitrogen flux in leaf litter degradation investigated by protein-stable isotope probing (protein-SIP)

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The degradation of plant-derived materials like leaf litter is crucial for the complex mineralization cycles in nature. Consortia of bacteria and especially fungi greatly contribute to this key ecosystem process by expressing a suite of various extracellular and intracellular enzymes. In fact, these microorganisms decompose almost 90% of the plant biomass produced in terrestrial ecosystems but the keyplayers and the details of the carbon and nitrogen flux are widely unknown. By using ^{13}C and ^{15}N labeled plant material in combination with protein-SIP we were able to track the carbon and nitrogen flux and obtained a closer insight into the structure and function of a soil microbial community. Protein-SIP allows the identification of metabolically active species, interactions and metabolic networks by detecting and quantifying the incorporation of even little amounts like 1% of ^{13}C and ^{15}N into the proteins of the involved bacteria and fungi [1]. Soil from a tobacco field in Germany was homogenized and mixed with leaf litter from either ^{15}N-labeled tobacco or ^{13}C-labeled corn plants as substrate. The microbial growth within the approaches was monitored by measuring the biological oxygen demand up to 14 weeks. Sampling took place 7 times within the two week experiments and 9 times within the 14 week experiment. Proteins of the extracellular and the intracellular fractions were separated by 1D-SDS gel electrophoresis and peptides were analysed by UPLC coupled Orbitrap MS/MS. For protein identification the metagenome sequence of the soil from the tobacco field was conducted. 454 pyrosequencing resulted in ~390 Mb distributed over 871,000 reads with an average length of ~450bp. MG-RAST analysis showed that a large proportion of the functional genes belong to bacterial proteins (~97%) and to eukaryotic, especially fungal, proteins (~2%). For the ^{15}N tobacco litter experiment a total of ~10,100 peptides were identified to which the metagenome contributed with ~30%. Metaproteomic and protein-SIP data revealed changes in the composition of the microbial community over time. Relative isotope abundances in a range of ~10% to ~80% were detected with distinct clusters at ~60% and ~75%. Specific microbial orders such as *Actinomycetales*, *Rhizobiales* and *Pseudomonadales* showed a high metabolic activity and were designated as metabolic key players.

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PRMV002

Metaproteomics for process monitoring in environmental biotechnology

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Microorganisms represent a significant proportion of biomass on earth, drive many natural cycles and are applied in numerous biotechnological processes. So far nucleic acids are often used for identification of species and for characterization of metabolic potential in environmental samples. However with the progress in proteomics, extracellular and intracellular proteins can also be used as promising markers to describe biological activities of applied microbial communities.
A complete workflow for metaproteome analysis was established. It comprised protein extraction with liquid phenol, protein separation by SDS-PAGE or 2D-PAGE, tandem mass spectrometry (MS/MS) and the new software MetaProteomeAnalyzer for protein identification, quantification and statistical analysis of results. The workflow was applied for analysis of anaerobic digesters and wastewater treatment plants. Whereas conclusions of other metaproteomic studies were impaired by working with enrichment cultures or defined media, our workflow enabled access to samples from full scale plants.
Screening of ten anaerobic digesters on the level of SDS-PAGE revealed specific protein signatures containing similar and unique bands. These signatures showed changes that correlated with disturbances such as acidification. Enzymes involved in hydrolysis, in acidogenesis and in methanogenesis like methyl-CoM reductase were identified. The fact that the decrease of methyl-CoM reductase was found prior acidification could be used for biomarker development.
Sludge from wastewater treatment plants investigated by 2D-PAGE contained elastase 3A, which implied that the human serine protease was a persistent in wastewaters and could be used as marker for fecal contaminations in the environment. In addition, key enzymes of major metabolic processes in wastewater treatment plants (nitrification, denitrification and biological phosphor removal) were detected and could be applied as biomarkers, too. .
Although the proteomics approach described is too slow and too expensive for regular monitoring of wastewater treatment plants and anaerobic digesters, the correlation of proteomic data with process data gives valuable insights in metabolism of microbial communities and will support the identification of predictive biomarkers.

PRMV003

Quantitative proteomics of *Allochromatium vinosum*: insights into dissimilatory sulfur metabolism in a purple sulfur bacterium

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The purple sulfur bacterium *Allochromatium vinosum* DSM 180^T is a model organism for laboratory-based studies of oxidative sulfur metabolism. Here, we took advantage of the organism's high metabolic versatility and compared proteomic patterns under several sulfur-oxidizing conditions with that after photoorganoheterotrophic growth on malate using tandem mass tags for quantification.
With this global approach, 1505 of predicted 3302 proteins were detected by at least two peptides. Fifty of 60 proteins known or suggested to be involved in oxidative sulfur metabolism were identified by at least one peptide. Among these, the sulfide-oxidizing flavocytochrome *c* (FccAB), the substrate-binding protein SoxYZ of the thiosulfate-oxidizing Sox system and GarA were the most abundant, independent of the growth conditions. GarA is a peroxiredoxin/glutaredoxin hybrid and the protein from *Marichromatium gracile* exhibits glutathione amide-dependent peroxidase activity. In purple sulfur bacteria, *garA* resides immediately adjacent to *garB*, the gene for glutathione amide reductase. The most distinctive increase of protein abundance during growth on sulfur compounds was detected for the proteins of the DSR pathway which is essential for sulfur globule oxidation with values up to 28-fold for DsrB. Notably, the levels of the catalytic subunits of two membrane-bound complex iron-sulfur molybdoenzymes were also specifically elevated in the presence of sulfide and elemental sulfur. The first of these proteins is a periplasmically oriented putative polysulfide or sulfur reductase and we recently established that the second functions as a cytoplasmically oriented sulfite dehydrogenase (see Poster by Kesselheim and Dahl). The most prominent decrease in protein abundance in the presence of reduced sulfur compounds as compared to growth on malate was detected for the enzymes of assimilatory sulfate reduction.
In combination with a recent global transcriptomic approach, our comparative quantitative proteome analyses led to identification of several candidate proteins with appropriate subcellular localization and properties to participate in sulfur oxidation and will finally allow development of hypotheses for the sulfur oxidation pathways not only in *A. vinosum* but also in many other sulfur-oxidizing bacteria.

PRMV004

Ionophores calcimycin and ionomycin extract iron and manganese from soil bacteria - a potent strategy against competitors

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The ionophores calcimycin and ionomycin are secondary metabolites of terrestrial *Streptomyces* species [1, 2] and show potent antibacterial activity. Previously, the ionophore-mediated transport of individual metal cation species across model membranes was studied *in vitro*. These studies provided valuable insights into the transport mechanism - the ionophores can only cross membranes with a cation bound [3] - and into factors determining transport efficiency (cation complexation, translocation, and release rates) [4]. Two key questions, however, are yet unanswered: What is the antibacterial mode of action of these ionophores? And what is their ecological role?
To address these questions, we investigated the effects of calcimycin and ionomycin on metal ion homeostasis of the soil bacterium *B. subtilis* in chemically defined medium. Within 15 minutes of treatment, cells lost about 50% of their intracellular iron and manganese whereas calcium levels increased. Proteome analysis showed that *B. subtilis* manganese and iron depletion responses as well as oxidative stress responses are activated upon ionophore treatment. The generation of reactive oxygen species following ionophore treatment was confirmed employing a specific fluorescent probe.
In soil, iron availability is thought to be generally very low, and manganese can also be growth-limiting. Interestingly, in medium lacking iron and/or manganese, the sensitivity of *B. subtilis* to calcimycin or ionomycin increased by one to two orders of magnitude, likely because efflux of both is concentration-gradient dependent. Conversely, when no calcium was added to the medium, *B. subtilis* tolerated higher ionophore concentrations, suggesting that the lack of calcium in the medium either limits manganese and iron efflux or precludes harmful influx of excess calcium into the cells. Taken together, our findings suggest that ionophores act by extracting iron and manganese from bacterial cells. This is a potent antibacterial strategy against competing microbes, particularly in the soil, an environment rich in calcium and generally low in micronutrients.

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PRMV005

Thermotolerance in *Bacillus subtilis*: the role of oxidation in survival and heat induced aggregate formation

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The protective potential of the cellular protein quality control system and its regulation becomes evident in thermotolerance development, where cells, which are exposed to a short mild heat shock, are primed to survive a subsequent severe, otherwise lethal, heat stress. Using *B. subtilis* as a model organism, we investigated survival and protein aggregate formation during thermotolerance with wild type and mutant cells lacking heat shock regulators or chaperones and proteases.
We observed that *clpP* and *clpX* mutant cells exhibit an improved thermotolerance and increased survival during severe heat stress. Interestingly, this is accompanied by a strong reduction of heat induced cellular protein aggregates. We could demonstrate that accumulation of the transcriptional regulator Spx, which is subject to regulatory proteolysis by ClpXP, is responsible for the observed phenotype. The Spx regulon, which is known to be induced during oxidative stress, encodes proteins required for protection against thiol specific oxidative stress such as thioredoxin, a protein disulfide isomerase. Notably, artificial induction of a proteolysis-resistant Spx variant (SpxDD) *in trans* was sufficient to prevent heat induced protein aggregation and protect cells to some extent from severe heat stress. In addition, the artificial induction of thioredoxin also resulted in the prevention of heat induced protein aggregation.
This suggests that that protection from oxidative stress during heat shock in the cytosol plays an important role in prevention of protein aggregation. Consequently anaerobically grown *B. subtilis* cells were more protected against severe heat shock and much less protein aggregates were detected compared to aerobically grown cells. Our results clearly indicate that Spx is not only important for redox stress response but is also intricately involved in thermotolerance regulation and induced during heat shock response.
In the cytosol, proteins unfolded by heat could be prone to detrimental oxidation of thiol side chains resulting in disulfide bridges, which can stabilize non-native protein conformations or could promote covalent cross linking to other misfolded proteins. But in the absence of oxygen or in the presence of redox active enzymes repairing the unfavorable disulfide bonds, the stabilization and formation of protein aggregates promoted by oxidation can be prevented.

PRMV006

Total membrane complexome profiling of the anammox bacterium *K. stuttgartiensis*

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The anaerobic ammonium oxidizing (anammox) bacterium *Kuenenia stuttgartiensis* uses three reactions in its nitrogen catabolism, in which electrons are cycled through membrane-bound complexes, thereby establishing a proton motive force. In the genome there is a high redundancy of genes that encode proteins and protein complexes that are involved in the electron transport chain (ETC)[1]: 4 ATP synthase clusters, 3 NADH dehydrogenase clusters and 3 bc_1 complexes, that all show variations to the canonical bacterial bc_1 complex[2,3]. The whole cell proteomic approach[2] did not reveal whether or not all complexes are actually expressed and functionally present in the membranes of *K. stuttgartiensis*.
Here, we present the first whole membrane complexome of *K. stuttgartiensis*, with focus on the ETC complexes. For this purpose, we applied two-dimensional gel electrophoresis, followed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS)[4]. Intact membrane complexes were loaded on a first dimension blue native (BN) PAGE, allowing separation by molecular mass. This gel lane was either applied to a second dimension SDS PAGE for MALDI-TOF analyses of the protein spots, or cut into equally sized slices and analyzed via LC-MS/MS. Using protein abundance profiling techniques, we were able to detect and estimate all complexes associated with the membranes. In future, this study will form a solid basis for new research questions, can supplement subcellular localization studies and, coupled to activity assays can assist in purification protocols.

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PRMV007

Lipidomics and proteomics identifies oleaginous yeast-like behavior and two novel acetyltransferases in tetraacetyl phytosphingosine producing yeast *Wickerhamomyces ciferrii*

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The Wickerhamomyces ciferrii strain NRRL Y-1031 F-60-10A is a well-known producer of tetraacetyl phytosphingosine (TAPS) and used for the biotechnological production of sphingolipids.
Question: In this study sphingolipid metabolism was explored by employing a dual platform mass spectrometry strategy.
Methods: The first step comprised metabolic 15N-labeling in combination with label-free proteomics using high resolution LTQ Orbitrap mass spectrometry. Next, selected reaction monitoring tandem mass spectrometry provided targeted quantification of sphingoid base biosynthesis enzymes.
Results: In total, 1697 proteins were identified and 123 enzymes of the central metabolism were differentially expressed. From the proteome point of view, TAPS producer strain NRRL Y-1031 F-60-10A displays oleaginous yeast-like behavior and possesses a 2-fold higher abundance of eight sphingolipid biosynthesis enzymes.
Importantly, TAPS-synthesizing acetyltransferases were identified. Using deletion mutants, results at the sphingolipid metabolic phenotype level confirmed the catalytic role of identified acetyltransferases. Acetylation of phytosphingosine in W. ciferrii is catalyzed by acetyltransferases Sli1p and Atf2p, encoded by genes similar to Saccharomyces cerevisiae YGR212W and YGR177C, respectively. Liquid chromatography-coupled triple stage quadrupole mass spectrometry confirmed an almost complete loss of tri- and tetra-acetyl phytosphingosines upon ablation of SLI1, whereas the loss of ATF2 resulted in a 15-fold increase in triacetyl phytosphingosine. Most likely, it is the concerted action of these two acetyltransferases that yields TAPS. Sli1p catalyzes initial O- and N-acetylation, producing triacetyl phytosphingosine, to be acetylated subsequently by Atf2p, yielding TAPS.
Conclusions: The current study demonstrates that dual platform mass spectrometry-based proteomics can be employed to identify key steps in ill-explored metabolite biosynthesis pathways of non-conventional microorganisms. Identification of phytosphingosine as substrate for alcohol acetyltransferase Atf2p broadens the known substrate range of this enzyme and may be exploited in future to enhance the secretion of heterologous compounds.

PRMV008

Pupylation in the biotechnological workhorse *Corynebacterium glutamicum*

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It has been described previously that mycobacteria tag proteins with the prokaryotic ubiquitin-like protein (Pup) for degradation *via* the proteasome [1, 2]. Activated Pup is attached to target proteins forming an isopeptide bond. Global approaches to identify pupylated proteins ("pupylomes") have been published for *Mycobacterium tuberculosis* and *M. smegmatis* as well as for *Rhodococcus erythropolis* [3-6]. Interestingly, genes known to be involved in pupylation are also present in other actinobacteria, which do not possess a proteasome. Amongst these resides *Corynebacterium glutamicum*, a workhorse in biotechnology, which is used in the production of L-glutamate (2.1 million tons/year) and L-lysine (1.5 million tons/year). We analyzed the pupylome of *C. glutamicum* by multidimensional protein identification technology (MudPIT). More than 50 pupylated proteins including a variety of enzymes of central and amino acid metabolism were identified and compared with the mycobacterial pupylomes to deduce common target proteins. In addition, global transcriptome and proteome analyses of a pupylation-defective *C. glutamicum* strain were performed. The results help to elucidate the role of pupylation in *C. glutamicum*.

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PRMP001

Metabolism of the polythioester precursor substrates 3,3'-thiodipropionic acid and 3,3'-dithiodipropionic acid and their influence on the proteome of *Ralstonia eutropha* H16

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Polythioesters (PTEs) are non-biodegradable biopolymers that can become of great value for medical or technical applications requiring durable materials. *Ralstonia eutropha* H16, the model organism for storage of poly(3-hydroxybutyric acid), is capable of utilizing 3,3'-thiodipropionic acid (TDP) and 3,3'-dithiodipropionic acid (DTDP) as precursor substrates for biosynthesis of copolymers consisting of 3-hydroxybutyric acid and 3-mercaptopropionic acid (3MP) . To elucidate the catabolic pathways of TDP and DTDP *in R. eutropha* H16, extensive functional genome analyses using DNA microarrays were applied. Based on these data several defined deletion mutants of *R. eutropha* H16 were generated and the resulting effect on polythioester accumulation was investigated. In a second approach, proteomic analyses were employed to analyze the impact of TDP and DTDP on the proteome of *R. eutropha* H16. Thereby, proteins involved in transport as well as enzymes putatively catalyzing the cleavage of the two organic sulfur compounds could be identified. Deletion of the *Bordetella* uptake gene (Bug)-like periplasmic solute receptor *bugI* caused significant differences in the polymer composition during growth on DTDP: the wild type accumulated copolymers with a 3MP content almost four times higher than the content of the deletion mutant *R. eutropha* Δ*bugI*. Hence, an involvement of this substrate binding protein in DTDP uptake is conceivable as Bug proteins were previously shown to interact with primary and secondary transport systems. Furthermore, the thiol-disulfide interchange protein DsbD and the thiol-disulfide isomerase FrnE might be involved in the reductive cleaving reaction of DTDP into two molecules 3MP. 3MP, which is the common cleavage product of TDP and DTDP, induces intracellular oxidative stress leading to the increased expression of different enzymes of the cellular stress response, like the chaperones HchA and GroL, several peroxidases and the glutathione *S*-transferase. By these analyses, the TDP and DTDP metabolism in *R. eutropha* H16 should be unraveled to enable the biosynthesis of designer made PTEs with different 3MP contents, which strongly influence the polymer features.

Peplinski K, Ehrenreich A, Doring C, Bomeke M & Steinbüchel A (2010) *Appl Microbiol Biotechnol* **88:**1145-1159.

PRMP002

High-throughput analysis of stable isotope probing experiments in metaproteomic studies

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Stable isotope probing[1] (SIP) has become one of the most suitable methods to identify metabolic key player in complex microbial communities. The introduction of stable isotopes into the biomass via the degradation of labeled substrates of interest makes microbial ecology independent of isolation and thus reduces the risk of biases. This risk is further reduced when the SIP experiment is directly performed *in situ*. SIP is well established for DNA/RNA and fatty acids, but the later lacks the phylogenetic resolution and the former needs incorporation rates of at least 20 atom%. Both give no further clue about fluxes or physiology.
SIP with proteins (protein-SIP[2]) combines the power of metaproteomics whose protein identification show the phylogenetic origin of proteins and the metabolic network which is actually active. Furthermore the SIP part allows identification of incorporation of heavy isotopes (usually ^{13}C or ^{15}N) into the proteins. This incorporation can be quantified accurately and does not only prove substrate usage, but also to which extent. Additionally the ratio of unlabeled and labeled proteins can be used as a measure for turnover and induction. The isotopic distribution itself can give clues about the direct use of a substrate or of degradation products from another organism[3].
Besides the many advantages of protein-SIP, the evaluation of mass spectrometric data is at this point an enormous bottleneck. Spectra have to be checked manually one at a time. This results in months of tedious evaluation. We now present a bioinformatics solution which reduces the time of analysis to days and even supports the evaluation of the received information. The Protein-SIP tool is embedded in the frame of OpenMS[4] and does recognize and calculate relative isotope abundances and labeling ratios. For further evaluation it is able to pre-cluster the identified proteins into groups of similar incorporation behavior and produces a convenient but complete report of results.
The final version will drastically improve the convenience of protein-SIP experiments.

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PRMP003

Proteomic and metabolomic analyses of amino acid degradation pathways in *Phaeobacter gallaeciensis* DSM 17395

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Phaeobacter gallaeciensis DSM 17395 is a nutritionally versatile model organism for the marine alphaproteobacterial *Roseobacter* clade. This clade accounts for up to 25% of the marine bacterioplancton in the oceans and therefore plays a prominent role in mineralizing organic material. This material consists to a major part of proteins which are hydrolyzed to amino acids. *P. gallaeciensis* grows with amino acids, but the degradation pathways are largely unexplored. They were manually reconstructed from the annotated genome. For further functional analysis nine amino acids were selected, whose degradation involves multiple, in parts unclear steps: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. In addition, succinate was chosen as a reference state. *P. gallaeciensis* was grown in a defined salt water medium with one of the selected amino acids or succinate as sole carbon source. Cells were harvested at half-maximal OD_{600} for proteomic (2D DIGE, shotgun, membrane proteomics), metabolomic and enzyme activity analyses. In total 1,503 different proteins were detected, 463 of which showed changes in abundance as resolved by 2D DIGE. For each of the selected amino acids, specific degradation enzymes were up-regulated. In addition, metabolome analyses revealed up to 110 identified metabolites of which some are specific for the suggested amino acid degradation pathways.
The analyses were complemented with enzyme activity measurements, to further our understanding of the degradation pathways for the studied nine amino acids in *P. gallaeciensis*.

Key words
Roseobacter clade, amino acid degradation, 2D DIGE, metabolomics, enzyme activity, regulation

PRMP004

Towards the cell envelope proteome of *Phaeobacter gallaeciensis* DSM 17395

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Phaeobacter gallaeciensis DSM 17395 is a gram-negative marine bacterium belonging to the ecological important alphaproteobacterial *Roseobacter* clade. Gram-negative bacterial cells consist of five main compartments: (i) cytoplasm, (ii) cytoplasmic or inner membrane, (iii) periplasm, (iv) outer membrane and (v) extracellular space. The protein composition of each subcellular fraction was analyzed, including substractive proteomics, to define the cell envelope proteome. Thus, besides protein abundance profiling a reliable overview of the cell architecture on a proteomic scale (e.g. visualization of the outer membrane proteome) was achieved. Additionally, information about protein targeting (protein secretion) could be provided. Notably, protein secretion, under the tested conditions, is dominated by the major extracellular hemolysin/RTX-like proteins, encoded by PGA1_c26140 and PGA1_65p00350. The established approach provides the basis for physiological studies of cell envelope proteins and facilitates analysis of protein sorting/targeting, supporting systems biology of the ecological relevant model organism *P. gallaeciensis*.

Key words
Phaeobacter gallaeciensis, subcellular proteomics, outer membrane proteome

PRMP005

The role of ATP-dependent proteases in *Burkholderia cenocepacia* biofilm formation

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Strains of the *Burkholderia cepacia* complex (Bcc) are a heterogeneous group of bacteria that are naturally found in the soil and rhizosphere of plants. However, some members like *B. cenocepacia* can cause life-threatening lung infections in patients suffering from cystic fibrosis (CF) or in immune-compromised persons. Most of the infections take place in form of bacterial biofilms which display an even worse threat because of their capacity to resist classical treatments with antibiotics. Mutants defective in ATP-dependent proteolysis were shown to have decreased biofilm formation capacity. Here, we use a mass spectrometry-based approach (LC/IMS^e) for label-free quantification of the cytoplasmic proteome and to elucidate the role of the ATP-dependent proteases Clp and Lon in biofilm formation of *B. cenocepacia* H111.

PRMP006

Quantity of proteins in outer membrane vesicles and outer membrane exemplified on uropathogenic *Escherichia coli*: A comparative approach

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Gram negative bacteria release outer membrane vesicles (OMVs) to the extracellular milieu. These small spherical blebs are mainly composed of the outer membrane as well as periplasmatic proteins. Because of the characteristic impact of OMVs in mechanisms of pathogenicity, bacterial stress response and working as innate bacterial defense against bacteriophages and antimicrobial peptides, the OMVs are moving more than ever into the focus of interest: "*The diverse abilities of OM vesicles to modulate immune responses, deliver toxins and other virulence factors to host cells, and aid in biofilm formation all attest to the importance that these secreted elements can have in bacterial pathogenesis.*" ***[1]***
In this work a mass spectrometry based gel free approach (LC/IMS^e) was used for a label free absolute quantification on a global scale. The emphasis of this investigation is particularly given to analyzing proteins in OMVs produced by uropathogenic *E. coli* CFTO73 during early stationary phase. By additional quantification of the outer membrane the proteins, the results represent the first quantitative comparison between proteins in both fractions of *E. coli*.
About 100 quantified proteins were unique or significantly enriched in the OMVs, including lipoproteins and known virulence factors. Additionally, a large number of proteins from prophages as well as proteins with unknown functions were identified only in the OMV fraction. These findings allow the presumption of producing phage proteins and bacterial proteins with unknown function by *E. coli* CFTO73, which are possibly involved in its virulence. It gives further hints to the effect of the innate bacterial defense system in *E. coli* CFTO73 using OMVs to protect against released bacteriophages.

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PRMP007

A metaproteomic approach to unravel the role of procaryotes in the lichen *Lobaria pulmonaria*

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Lobaria pulmonaria is a lichenized fungus which grows epiphytic on trees in large, leaf-like thalli. It has two autotroph symbionts, called photobionts, the green alga *Dictyochloropsis reticulata* and a cyanobacterium which belongs to the genus *Nostoc*. The alga fixes carbon dioxide, while the cyanobacterium fixes atmospheric nitrogen. Next to *Nostoc sp*, there are numerous other prokaryotic organisms living on the thalli. A recent metaproteomics study revealed that α-Proteobacteria are dominating the bacterial community (Schneider et al., 2011). Our current metaproteomic approach aims on a detailed analysis of the functionality of prokaryotes in the lichen *L. pulmonaria*. More specifically, we would like to understand if prokaryotes act as mutualistic or commensal partners, or even as parasites within the lichen community. To this end, we have analyzed the lichen metaproteome from old and young thalli by a combination of one-dimensional gel electrophoresis and liquid chromatography coupled to tandem mass spectrometry (GeLC-MS/MS). Proteins were assigned to phylogenetic and functional groups as well as quantified by spectral counting by a newly developed bioinformatics workflow (PROPHANE). Until now, 326 proteins have been reliably assigned to different phylogenetic and functional groups. Most of these proteins (301) were detected in both parts of the lichen thalli; however, 24 proteins were only found in the young and one protein was exclusively detected in the old thalli. In the young thalli 77 %, 9%, and 7%of the proteins could be assigned to fungal, algal and bacterial origin, respectively. Interestingly, the amount of fungal proteins seems to be higher in the older thalli, where 87 % of the proteins could be assigned to fungal origin. The majority of the bacterial proteins were produced by a-proteobacteria, which is in good accordance to our previous findings. To complement our proteomics analyses, the composition of the bacterial community will also be analyzed by 16S rDNA sequencing.

Schneider, T., Schmid, E., de Castro, J.V., Jr, Cardinale, M., Eberl, L., Grube, M., Berg, G., and Riedel, K. (2011). Structure and function of the symbiosis partners of the lung lichen (Lobaria pulmonaria L. Hoffm.) analyzed by metaproteomics. Proteomics *11*, 2752-2756.

PRMP008

Elucidation of Linezolid stress response in *Staphylococcus aureus* with absolute and relative protein quantification

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Linezolid is an important antibiotic of last resort against the gram-positive pathogen *Staphylococcus aureus*. Although the general mechanism by which Linezolid affects the protein synthesis is already described, little is known about the physiological consequences on protein level in the cell. We observed temporary growth arrest in *S. aureus* USA300 FPR_3757 after Linezolid stress, followed by resumption of growth although Linezolid was still present in the media.

We induced Linezolid stress in growing *S. aureus* USA300 cells and performed a protein analysis at three time points after Linezolid stress and analysed two control samples. Absolute quantitative data was obtained by a Hi3 IMSE approach. In addition to the analysis of cytosolic proteins, we applied a newly developed workflow for the absolute quantification of extracellular proteins. This allowed us for the first time to monitor changes in absolute protein abundances of cytosolic and extracellular proteins including virulence factors. As we observed dramatic changes in cell size and shape, we analysed them with scanning electron microscopy and laser fluorescence microscopy.
By combination of these approaches we achieved a comprehensive view on the physiological impact of Linezolid on *S. aureus* USA300_FPR3757, a widespread high virulent MRSA strain. We could identify about 50% of the predicted proteins. Complete quantitative data for all time points could be achieved for 30% of all proteins. Our data shows that Linezolid induces systemwide changes in protein abundance affecting ribosomal proteins, virulence factors and proteins of the main metabolic pathways like the TCA cycle. For 37% of all quantified proteins significant changes in abundance were observed.
We are convinced our data will improve the general understanding of the Linezolid mode of action on pathogenic bacteria.

PRMP009

The proteome response to folate biosynthesis inhibitors sulfanilamide and trimethoprim provides first insights into purine biosynthesis regulation by intermediates of folate biosynthesis in *Bacillus subtilis*

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Folate is essential for all organisms, because tetrahydrofolate is a crucial co-factor that accepts and releases C_1-units in numerous biological reactions. Specifically two enzymes of the purine biosynthesis require this co-factor for generation of inosine monophosphate. Purine biosynthesis regulation has been studied in *Homo sapiens* and it has been found that dihydrofolate competitively inhibits the purine biosynthesis enzyme aminoimidazole carboxamide ribonucleotide (AICAR) formyltransferase causing AICAR accumulation [1, 2]. In *Saccharomyces cerevisiae* this intermediate increases the transcription of purine biosynthesis genes [3]. To investigate the influence of folate biosynthesis inhibition on purine biosynthesis in *B. subtilis* we used the antibiotics trimethoprim and sulfanilamide targeting two reactions in folate biosynthesis. Sulfanilamide inhibits dihydropteroate synthase and trimethoprim prevents formation of the active co-factor tetrahydrofolate from dihydrofolate two steps downstream.
Proteome analyses of proteins newly synthesized in response to both antibiotics result in clearly distinct proteome profiles. Interestingly, synthesis of purine biosynthesis enzymes is down-regulated under sulfanilamide stress. In contrast, synthesis of histidine and purine biosynthesis enzymes is upregulated in response to trimethoprim. While both antibiotics lead to a decrease in the final product tetrahydrofolate, we assume that its direct precursor dihydrofolate accumulates only due to dihydrofolate reductase inhibition by trimethoprim resulting in differential regulation of purine biosynthesis. Both antibiotics induce the σ^B-dependent general stress response, probably due to a drop in ATP and GTP levels known to activate σ^B in response to energy limitation [4]. Sulfanilamide is the stronger stimulant of the σ^B-dependent response.
We could show that sulfanilamide and trimethoprim stress result in different proteome responses. From these data we propose that dihydrofolate, expected to accumulate due to trimethoprim treatment, regulates purine biosynthesis in *B. subtilis*.

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PRMP010

Multiple Reaction Monitoring Assay for Reductive Dehalogenases of *Dehalococcoides mccartyi* sp. CBDB1

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Chlorinated hydrocarbon pollutions pose a threat to nature and human health due to their toxic and cancerogenic potential. The ability of bacteria belonging to the genus *Dehalococcoides* to use a broad range of chemicals from this class as terminal electron acceptors shows potential for bioremediation. The strictly anaerobic *Dehalococcoides mccartyi* strain CBDB1 utilizes a wide range of electron acceptors with the help of its reductive dehalogenase enzymes. In the sequenced genome 32 different reductive-dehalogenase-homologous (*rdh*) gene operons are annotated [1], from which 13 were detected on the proteome level. This high count sparks a special interest in the differences between the gene products. One possible explanation would be strong substrate specificities of the encoded reductive dehalogenases. To analyse possible substrate dependent differences in protein quantities of expressed Rdhs, the proteome of bacteria cultivated under identical growth conditions but with different electron acceptors was analysed.
First a shotgun proteomics based approach using liquid chromatography coupled tandem mass spectrometry (LC-MS/MS) was carried out in order to get an overview of the global proteome of CBDB1. Based on the results, a multiple reaction monitoring (MRM) method employing stable isotope labelled standards is being developed to enable an absolute quantification of Rdh protein amounts. For a first MRM assay, six reductive dehalogenase proteins prominent in shotgun measurements were chosen together with three housekeeping proteins as control. Proteotypic peptides were selected according to their properties matching MRM demands and detectability in shotgun measurement. For each peptide three transitions were defined. As improvement of the method, differing retention times of the peptides will be used to establish a scheduled MRM Assay. Results from reliable quantification of the predominantly transcribed Rdh proteins for different substrates will further improve the understanding of the correlation between electron acceptors provided during cultivation and expression of *rdh* enzymes by CBDB1.

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PRMP011

The phosphoproteome of *Staphylococcus aureus*

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As causative agent of a high number of nosocomial infections, *Staphylococcus aureus* emerged as an important human pathogen. Though reversible protein phosphorylation is a major mechanism in the regulation of fundamental signaling events in bacterial cells, only limited information on protein regulation by means of phosphorylation events are available for this pathogen. *S. aureus* has been shown to possess a repertoire of protein kinases and phosphatases. Therefore, further investigations of the staphylococcal phosphoproteome lead to a better understanding of cell physiology, deciphering molecular and cellular mechanisms that underlie pathogenesis.
S. aureus COL has been shown to possess the protein PtpB which is assumed to be an arginine phosphatase. By investigating the deletion mutant Δ*ptp*B, in comparison with the wild type, we tried to analyze the changes in the phosphoproteome focused on the phosphorylation on arginine residues. Whilst taking into account the reduced stability of arginine phosphorylations, we therefore modified the methods of Olsen and Macek

2009 (1) as well as Elsholz *et al*. 2012 (2). In revealing several target proteins phosphorylated on arginine residues, first results pave the way to conclusive identification and characterization of the staphylococcal phosphoproteome.
Additionally, we established a gel-free approach to quantify phosphorylated proteins. Quantification of the phosphoproteome was accomplished in combining metabolic labeling with the enrichment of phosphopeptides using SCX chromatography and TiO_2-based purification followed by high-resolution LC-MS/MS. To analyze the influence of the serine/threonine-phosphatase Stp, we compared the phosphoproteome of the *S. aureus* NEWMAN wild type with the deletion mutant Δ *stp* in a quantitative manner. Although further investigations have to confirm the preliminary results, several proteins phosphorylated on STY-residues could be quantified.

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PRMP012
Phosphoproteome analysis of the Archaeon *Sulfolobus acidocaldarius* with a special focus on central carbohydrate metabolism

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Posttranslational modifications are of major interest for the regulation of cellular processes. Reversible protein phosphorylation is the main mechanism to control the functional properties of proteins in response to environmental stimuli [1]. In the 80's protein phosphorylation has been demonstrated in the third domain of life, the Archaea [2]. So far only few archaeal protein kinases (PKs) and protein phosphatases (PPs) were investigated and informations regarding signal transduction cascades are very limited. Bioinformatic analysis revealed that bacterial-type two- and one-component systems are present in the euryarchaeota (e.g. CheA/CheY in *Halobacterium salinarium*) [3] but are absent in the creanarchaeota [1]. In contrast eukaryal-type PKs and PPs are found in all archaeal kingdoms indicating that the eukaryal-type protein (de)phosphorylation system plays a major role in Archaea. Investigations of archaeal phosphoproteomes are scares and so far only the phosphoproteomes from *S. solfataricus* [4] and *H. salinarium* [5] have been analyzed.
Model organism of this study is the thermoacidophilic Creanarchaeon *Sulfolobus acidocaldarius* [6]. Bioinformatic investigation revealed that *S. acidocaldarius* harbors twelve PKs and two PPs [1]. In order to get first insight into archaeal signal transduction deletion strains of both PPs were constructed. Three *S. acidocaldarius* strains (MW001 (background strain), *Δptp* and *Δppp*) were investigated by using the gel and enrichment free precursor acquisition independent from ion count (PAcIFIC) approach [7]. We identified a very high number of phospho-proteins with an unusual pS/pT/pY%-ratio compared to *H. salinarium* and other prokaryotic phosphoproteomes. The data will be presented with a special focus on the central carbohydrate metabolism.

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PRMP013
Evaluation of multidimensional separation techniques to increase identification of proteins from anaerobic digesters

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Biogas production from agricultural crops and waste is a main source for renewable energies in Germany. In anaerobic digesters a complex microbial community converts biogenic waste to biogas. Unfortunately, disturbances of the biogas process occur and cause economic losses. Besides technical failures the microbial community itself was often identified as a reason for process instability. To improve performance and efficiency of digesters, a deeper knowledge of the physiological status of the microbial community is required and biomarkers for recording of process deviation or prediction of process failure have to be identified.
Previous works based on 2D-electrophoresis demonstrated that the analysis of the metaproteome [1, 2] provided insights into the metabolisms of the microbial community. Using SDS-PAGE with subsequent mass spectrometry, stable protein patterns were detected in most anaerobic digesters. Furthermore, it could be shown that severe changes, e.g. acidification resulted in significant changes in the proteome.
However, the high complexity of metaproteomes derived from anaerobic digesters was still a challenge. In this study, different combinations of separation techniques to reduce complexity of proteomic biogas plant samples were compared regarding the subsequent identification of proteins by tandem mass spectrometry (MS/MS). (i) One-dimensional: proteins were tryptically digested and the resulting peptides were separated by reversed phase chromatography prior MS/MS. (ii) Two-dimensional: proteins were separated by SDS-PAGE according to proteins molecular weights before tryptic digestion (iii) Three-dimensional: gel-free fractionation by isoelectric point (IEF) was added before SDS-PAGE.
A comparison of two anaerobic digesters operated at mesophilic or at thermophilic conditions was taken as example. Adding further dimensions of separation before protein identification by MS/MS increased the number of identified proteins but also the experimental work and time for measuring the samples. The high resolution of the three-dimensional approach allowed a clear distinction between the microbial communities at mesophilic or at thermophilic conditions. However, regarding a broad screening of anaerobic digesters for process biomarkers it is substantial to find a balance between the experimental efforts and analytical resolution.

PRMP014
MetaProteomeAnalyzer: A software tool for functional and taxonomic characterization of metaproteome data

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The functional analysis of highly complex microbial communities is still a challenging task in proteomics. However, knowledge obtained by metaproteome analysis may be applied to improve performance and cost-efficiency of many bioprocesses including anaerobic digestion, waste water treatment, and remediation of contaminated soil and water. Furthermore metaproteome analysis can also be applied to investigate the complex interactions in the human gut.
Currently, analysis and interpretation of data derived from LC-MS/MS experiments present a major bottleneck in metaproteomics. In contrast to pure-culture proteomics, metaproteome samples are heterogeneous and more complex. Moreover, for the major part of the microorganisms, protein sequence information is not available resulting in a low protein identification rate. To overcome some limits of existing approaches, the software tool MetaProteomeAnalyzer (MPA) was being developed for functional and taxonomic characterization of metaproteomics data.

Besides an advanced protein identification by a combination of multiple database search algorithms (Crux, OMSSA, X!Tandem, Inspect) and a spectral library search, the identification of proteins from unsequenced species by de novo sequencing and a BLAST search are included in the workflow. In addition, features for the analysis of the taxonomic diversity of a microbial community and for the functional classification of proteins are available.
The performance of MPA was demonstrated by the analysis of metaproteome data obtained from full-scale anaerobic digesters. Proteins involved in methanogenesis were identified and plotted into pathway maps. The key enzyme for methanogenesis, methyl CoM reductase, was taxonomically assigned on genus level allowing the estimation of the contribution of acetoclastic and hydrogenotrophic methanogens to the reactor performance.

PRMP015

Unraveling the biosynthetic pathway of the new volatile sodorifen of *Serratia* sp. by 2D-Gel analysis

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Serratia species are Gram-negative facultative anaerobic, rod-shaped gamma-proteobacteria (Enterobacteriaceae). They appear ubiquitously in soil, water, plants and animals, including humans, and many of the species emit characteristic blends which smell fishy/urinary or musty/potato-like. The potential of volatile (VOC: volatile organic compound) emission was recently rediscovered for *Serratia* as well as for other bacteria. At the present state of the art ca. 800 different volatiles were documented to be released from ca. 250 bacterial species (1). Approximately 100 compounds were found in the headspace of *S. odorifera* 4Rx13 (2), an isolate obtained from the rhizosphere of *Brassica napus* (3). The composition of volatile blends of the bacterium was altered when grown on complex medium or minimal medium supplemented with different combinations of amino acids and carbon sources. Most notably is the major compound sodorifen, which has an unusual and unique structure. To unravel its unknown biosynthetic pathway we performed various experimental approaches, including differential proteome analysis of high or low sodorifen production. A computer assisted 2D-gel analysis with “SameSpots” was used to identify differential proteins, which may include sodorifen biosynthesis enzymes. Candidate proteins were picked and analyzed with MALDI-TOF/MS.

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PRMP016

Cluster analysis of MALDI-TOF mass spectra and GC-MS of long chain fatty acids reveals environmentally related similarities in *Pseudomonas aeruginosa* strains isolated from various technical water systems

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Introduction: *P. aeruginosa*, an opportunistic pathogen, can be found worldwide in the liquid phase or in biofilms of various types of technical water systems. Little is known about the genotypic and phenotypic variability of this species in this environment. In a survey, 77 strains of *P. aeruginosa* isolated from various technical water systems and seven reference strains showed no correlation of their phenotypic traits with genotypic traits ([PFGE] pattern of SpeI restricted genomic DNA) or with the origin of the isolate. Thirty-nine different clonal variants could be identified by PFGE. To answer the question whether MALDI-TOF mass spectrometry of bacterial proteins or the gas chromatographic identification of bacterial long-chain fatty acid patterns can contribute to a further differentiation of these isolates at the subspecies level and can reveal any similarities related to the environmental origin of the strains, all 175 strains were analyzed by MALDI-TOF MS and 84 by GC-MS too.
Materials and Methods: From fresh cultures on Columbia agar bacteria were smeared on two sample positions on the MALDI-target at a time. After adding DHB as organic matrix spectra were generated with a Voyager-DE STR™ mass spectrometer and identified by using the SARAMIS™ database. The gas chromatographic determination of the bacterial long-chain fatty acid pattern was performed on a Hewlett-Packard GC System (5890 Series and [MIS] MIDI). All peak lists of MALDI-TOF mass spectra and separately of GC-MS were used for an agglomerative hierarchical cluster analysis and a principal component analysis with the software DataLab™.
Results: MALDI-TOF peak lists clustered mainly if the strands were clonically identical. In some cases, strands of different clonal variants clustered if they had been isolated from similar water systems. There was no overall correlation between clusters based on MALDI-TOF MS and those based on GC-MS of fatty acid patterns.
Discussion: Raw MALDI-TOF mass spectra contain many more peaks than necessary for the identification of microbial isolates based on the data base matching of peaks selected for maximum species specificity. Considering all peaks within the fingerprint range of 2000 to 20,000 Da allows the classification of *P. aeruginosa* strains in respect of their environmental origin in some cases which is not possible by genotyping or phenotyping based on established methods. This offers new options for the analysis of the influence of environmental conditions on the bacterial proteome.

PRMP017

Metabolomic analysis of the switch between acidogenesis and solventogenesis in continuous cultures of *Clostridium acetobutylicum*

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Despite the long history in using *C. acetobutylicum* for the production of butanol, still little is known about the regulation of the metabolic shift from acids to solvents. It is the goal of the collaborative systems microbiology project ´COSMIC2` to increase the knowledge on the key regulatory and metabolic events that occur during the shift. Especially key metabolites, like butyryl-phosphate and acetyl-phosphate, but also other glycolytic intermediates may play a crucial role. A set of knockout strains are being developed and their fermentative metabolism is analysed by transcriptomics, proteomics and metabolomics. While the analysis of transcript levels and proteins is rather straightforward, analysis of intracellular metabolites is still problematic. Especially the massive leakage of metabolites during the cold quenching of cells is a common problem in many metabolomics protocols. We compared various sampling techniques, quenching solutions and extraction methods. Cell leakage and metabolite recovery was determined by ATP measurements. The developed metabolomics protocol was used to analyse the metabolites from steady state acidogenic and solventogenic cells, as well as cells from different points of the intermediate shift. A targeted set of metabolites was analyzed and quantified by validated LC-MC methods (ion-pair/orbitrap MS). The results of the development of the metabolomics protocol and the first metabolite data will be presented.
The COSMIC2 project is carried out in close collaboration with research groups in Berlin, Munich, Rostock, Ulm and Nottingham.

PRMP018

Dynamics of amino acid utilization by *Phaeobacter gallaeciensis* DSM 17395

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Phaeobacter gallaeciensis DSM 17395, a model organism for the marine alphaproteobacterial *Roseobacter* clade, is capable of using a broad range of different organic carbon sources, favouring proteinogenic amino acids, their derivatives and peptides. The organism is well adapted to the low nutritional marine environment; being associated with algae, it can profit from the release of organic matter by the collapse of seasonal phytoplankton blooms. To study the dynamics of the uptake and metabolism of these substrate-mixtures, *P. gallaeciensis* was cultivated in an artificial saltwater medium containing a mixture of amino acids (casein hydrolysates) as sole carbon source.
While almost all of the 15 detected amino acids were utilized, they could be grouped according to their specific depletion rate. A time-resolved integrated proteomic (2D DIGE, shotgun, membrane proteomics) and metabolomic approach (GC-MS) of *P. gallaeciensis* identified 1,655 different proteins and 121 (94 identified) metabolites, of which 180 proteins and 85 metabolites displayed altered abundance during cultivation. The analysis revealed that upon complete depletion of favoured amino acids the metabolism is quickly changed to enhance the uptake and catabolism of

alternative (less favoured) amino acids. The results of both proteome and metabolome analysis to be presented on the poster allows interesting insights into the various catabolic pathways.

Key words
Roseobacter clade, amino acid degradation, 2D DIGE, metabolomics

PRMP019

Impact of bovicin HC5 on the protein synthesis of *Staphylococcus aureus*

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Staphylococcus aureus is an opportunistic pathogen and the causative agent of a wide range of human diseases. In food industry, *S. aureus* causes great concern mainly due to the ability to adhere to surfaces and produce biofilms. Once contaminating foods, its growth can result in the production of enterotoxins which is considered the major cause of foodborne poisoning.
Bacteriocins are ribosomally synthesized peptides produced by Bacteria and some Archaea which can be used on controlling microbial growth in food.
In this study we investigate the effect of bovicin HC5 on the protein synthesis of *S. aureus* COL. This bacteriocin is produced by *Streptococcus bovis* HC5 and shows inhibitory effects on growth of different bacteria (Mantovani and Russell, 2003, De Carvalho, Vanetti and Mantovani, 2008).
The minimal inhibitory of bovicin (1.2 μM) on *S. aureus* COL cultivated in synthetic medium was similar to that of nisin (1.0 μM) - a bacteriocin which is already in use as a food preservative.
With the proteomic signature of bovicin treated *S. aureus* we hope to contribute to a better understanding of this bacteriocin and its effect on food-borne pathogens.

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PRMP020

^{13}C-metabolomics revealed that aerobic and anaerobic testosterone catabolism by *Steroidobacter denitrificans* proceed via highly divergent catabolic pathways

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The biodegradation of steroids is a critical metabolic process performed exclusively by bacteria. Although details of the aerobic steroids degradation are available, the pathways operating under anoxic conditions are yet to be unraveled. *Steroidobacter denitrificans* DSMZ18526, the model organism used in this study, has the unusual ability to degrade testosterone, regardless of the presence of oxygen. By adopting metabolomic approaches, we demonstrated that the model organism uses the 9,10-seco-pathway to degrade testosterone under oxic conditions. This pathway depends on the use of oxygen and oxygenases for oxygenolytic ring fission. In this case, the B-ring of testosterone was opened first, followed by *meta*-cleavage of the aromatic A-ring. Conversely, the detected degradation intermediates under anoxic conditions suggest a novel, oxygenase-independent testosterone catabolic pathway, the 2,3-seco-pathway, which differs substantially from the aerobic route. In the anaerobic pathway, the cleavage of the steroid core ring structure begins with the A-ring through a hydrolytic mechanism. Regardless of the growth conditions, testosterone is initially transformed to a common intermediate, 1-dehydrotestosterone. This intermediate is a divergence point at which the downstream degradation pathway is dictated by oxygen availability. The soluble protein patterns of *S. denitrificans* grown aerobically and anaerobically with testosterone differed considerably, indicating that this model organism uses distinct enzyme systems to degrade testosterone in the presence and absence of oxygen.

PRMP021

Metabolomics of CcpE-dependent activation of TCA cycle in *S. aureus*

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The tricarboxylic acid (TCA) cycle is a central metabolic pathway in aerobic organisms used to generate energy, biosynthetic intermediates, and reducing potential from the catabolism of carbohydrates, proteins and fats. In *Staphylococcus aureus*, transcription of TCA cycle genes is regulated by the catabolite control protein A (CcpA), CodY, and the respiratory response regulator SrrAB. Together, these regulators activate or repress transcription in response to changes in carbon, nitrogen, and oxygen availability.
Here we show that *S. aureus* produces a fourth TCA cycle regulator, NWMN_0641 (a member of the LysR family of transcriptional regulators with homology to CcpC of *B. subtilis*). Deletion of this regulator (tentatively named CcpE) in *S. aureus* markedly decreased the transcription of *citB* (encoding aconitase) and strongly reduced aconitase enzymatic activity in the mutant. Additionally, metabolomic studies (GC-MS and NMR-spectroscopy) demonstrated that inactivation of *ccpE* resulted in increased intracellular concentrations of acetate, citrate, lactate, and alanine, consistent with a redirection of carbon away from the TCA cycle. This result is also suggested by decreased intracellular concentrations of glutamate and glutamine in the *ccpE* mutant. Taken together, these data demonstrate that over-flow metabolism is increased due to a metabolic block in the TCA cycle at aconitase and not citrate synthase. In support of this conclusion, we observed that CcpE directly binds to the *citB* promoter but not to the *citZ* promoter in EMSA experiments.
In summary, we have identified a new regulator, CcpE, of TCA cycle activity in *S. aureus*.

PRMP022

Data analysis and management challenged by *in vivo* and meta-proteomics

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In recent years rapid developments of genomic and proteomic technologies allowed the analysis of protein mixtures with an extremely high sensitivity. At the same time the complexity of protein mixtures increased as for instance by analyzing environmental and tissue samples and body fluids. We developed different tools based on PHP and MySQL which support protein identification and protein expression analyses of such highly complex protein samples. (i) The efficiency of these proteomic studies largely depends on the protein sequence database used for peptide matching. We developed a tool called *f.suite* which is able to assemble different protein sequences (*e.g.* based on NCBI taxonomic ID) into one FASTA file. Moreover, different FASTA files can be combined and redundant sequences can be removed. (ii) Closely-related organisms are characterized by groups of proteins with identical peptide fragments and, hence, these proteins cannot be clearly distinguished by mass spectrometry. Such indistinguishable protein groups can be analyzed by *Prophane* which searches for possible commons of all proteins within one group on functional and taxonomic level. (iii) Extensive protein expression data sets can be stored in an integrative database system called *Aureolib*. This database system visualizes and links gene expression data derived from different experiments. To select specific expression profiles, *Aureolib* provides complex search and filter options.

PRMP023

Proteome analysis of *Verminephrobacter eiseniae*, the nephridial symbiont of the earthworm *Eisenia fetida*

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Lumbricid earthworms harbor extracellular symbiotic bacteria of the genus *Verminephrobacte*r in their excretory organs, the nephridia. This symbiosis is species-specific, with indications of a 100 million year-old host-symbiont co-speciation, and vertical transmission of the symbionts by deposition into the earthworm's egg capsules. Despite this intimate association, host and symbiont can be separated in the laboratory and cultured independently. This has allowed for comparative fitness experiments which revealed that the symbionts presence during embryonic development and in adult worms increases hatching success and shortens maturation time of the earthworms. However, even though several symbiont strains have been isolated and characterized to date, and their genomes are available, the exact physiological mechanism for this mutually beneficial symbiosis remains enigmatic.
Here we present the first proteomic data for *Verminephrobacter eiseniae*, the specific symbiont of the compost worm, *Eisenia fetida*, with the objective to investigate its metabolism during batch culture growth. Protein profiles of different growth phases were quantified by 2D-DIGE. Furthermore, analysis of the membrane enriched fraction together with whole cells shotgun by LC-ESI-MS provided identification of more than 1400 proteins. The physiological state of the batch cultures was compared to the *in situ* situation by quantifying cellular protein content and pre-rRNA expression. These results allow a first glimpse into the functional basis of the *Verminephrobacter*-earthworm symbiosis.

MIRRI001

The pan-European Initiative MIRRI: the microbial resource research infrastructure

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MIRRI builds a Pan-European distributed research infrastructure that provides microorganism services facilitating access to high quality microorganisms, their derivatives and associated data for research, development and application. It connects resource holders with researchers and policy makers to deliver the resources and services more effectively and efficiently to meet the needs of innovation in biotechnology.
Microorganisms and their derivatives provide essential raw material for biotechnology - but to date less than 1% of the estimated number of species are described and available to be harnessed by man. As new species are discovered, the expertise is difficult to locate to ensure their correct identification and this human resource is diminishing. Public sequence databases are expanding rapidly providing modern tools for identification but the information is often of poor quality and often not backed up by the biological material which would enable validation of data. The existing but fragmented resources, distributed across Europe, need to be coordinated and operated to common standards with facilitating policy. This can help focus activities to resolve key problems and address the big challenges in healthcare, food security, poverty alleviation and climate change.
Microorganisms play important roles in the environment and are harnessed for human benefit. These organisms are necessary for land reclamation, teaching and research. Integrated pest management programmes including the use of natural enemies to control pests and diseases reduce crop losses. Crop wastes are degraded and converted into compost or into useful products through the use of carefully formulated microbial communities. Microorganisms are being increasingly used in the bioremediation of industrial waste, and in biotechnological applications active molecular compounds are produced. Study of the ancestors of resistant disease organisms held in collections from times before antibiotics and other forms of chemical pollution will help provide solutions to public health problems.
There is a need for improved access to biological materials for research and better uptake into innovation. Gaps must be closed and important services must be covered.
For example, of 20,200 prokaryotic research strains in 835 articles in eight European journals in 2008 only 190 strains (0.94%) were deposited in public service collections. Coordinated policy to make such published but not deposited materials available is needed.
More information about the project structure and goals at www.mirri.org

MIRRI002

The means to achieve the goals of the Microbial Resource Research Infrastructure (MIRRI)

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MIRRI will ensure that microorganisms and associated data used in research are properly characterised, are of a quality that can be relied upon, and are exploited in such a way that delivers fair returns transparently to the places from which they originate. Got right, this effort will help underpin European jobs and economic growth through a more efficient bioindustry. It will help us better understand and exchange knowledge on microbial biodiversity. Significantly raising the quality of microbial science; ensuring reproducibility of data and better knowledge diffusion. MIRRI is in a critical preparatory phase where it will provide the tools and design to enter the construction and operational phases. Specifically it will establish the stakeholder governance mechanisms and an effective business plan to deliver and ensure longer term infrastructure sustainability. MIRRI needs to ensure the wide involvement of stakeholders to ensure that research needs are met and added value products delivered. There are a number of mechanisms to encourage input from users, policy makers and potential funders including a stakeholder forum (see www.mirri.org). MIRRI will work on improved transparency, tackling key obstacles to research needs in a co-ordinated way and work more closely with researchers. Ministries and science funding organisations are invited to join the consortium in order to ensure high quality research and good science and the protection of investment in publicly funded research. Mechanisms to focus cross infrastructure expertise and technology on user based requirements will be created; e.g. a distributed platform for microbial taxonomy to ensure best use of the remaining expertise. MIRRI will be open for all European resource holders meeting the threshold criteria particularly regarding quality of resources, data sharing and commitment to cross infrastructure services. Working with the user community associated information will be compiled to facilitate the uptake of these resources into research. The future will be to link this pan-European research infrastructure to other regional activities creating the Global BRC Network (GBRCN).
More information about the project structure and goals atwww.mirri.org

MIRRI003

The pan-European Initiative MIRRI: Needs and Impact

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More information about the project structure and goals atwww.mirri.org

MIRRI004

The pan-European Initiative MIRRI: Data Resources Management

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The Data Resource Management in MIRRI serves to improve the quantity, quality, interoperability and usage of data associated with biological material in Microbial Resource Collections (MRC) for stakeholders, in academia and industry. At present, no standardized protocols are available for submission of strain specific associated data (metadata) to MRCs resulting in heterogeneous and incomplete datasets. MIRRI will address this problem by developing concepts and standards for data acquisition. Common strategies for evaluation, curation, integration and interoperability of existing and future data across MRCs will be developed. Moreover, the requirements for data access will be investigated. This includes modules for a user-friendly interface and restricted user access assuring maximum data security. An assessment on existing tools, data-based platforms and standards and projects like CABRI, StrainInfo, YeastIP, Fungene, WDCM, Genomic Standards Consortium, Darwin Core, and GBRCN will be accomplished. All

developments will be conducted in close cooperation with other ESFRI infrastructure projects like the European Infrastructure of Open Screening Platforms for Chemical Biology (EU-Openscreen), the European Marine Biological Resource Center (EMBRC) and the European Life Sciences Infrastructure for Biological Information (ELIXIR) to avoid redundancy and duplication and exchange existing know-how. The described aspects will be addressed in MIRRI by four connected tasks: 1) data acquisition, 2) data evaluation and curation, 3) data integration and interoperability and 4) data access.
In summary, MIRRI will focus on the development of models and strategies to maximize the data quality and accessibility of data in MRCs, addressing the needs in academia and industry.
More information about the project structure and goals atwww.mirri.org

BTV1-FG
Biofilms for chemical production - an outlook

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There are several promising perspectives in relation to the use of biofilm systems for production of chemicals but also uncertainties concerning efficiency, reproducibility, controlability and population stability. Based on previous experience and results the presentation will point out some of the problems which must be solved, and discuss the potential of exploiting specific biofilm characteristics in designing biofilm communities for the production of chemicals, which are difficult to produce in more traditional fermentation processes.

BTV2-FG
Life and death in biofilms

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The common form of life of microorganisms takes place in aggregates, such as films at interfaces, flocs, sludges, granules or others, all of them usually called biofilms. In bio-films, the cells are embedded in a matrix of extracellular polymeric substances (EPS) which allow for the formation of microconsortia, gradients in the concentrations of oxygen, substrates, products as well as of pH-value and redox potential. A wide variety of habitats supports high biodiversity and makes biofilms the most successful (and oldest) form of life. In biofilms, photosynthesis evolved as well as biodegradation of organic materials; they are the carriers of the self-purification potential of soils, sediments and water.
They are also sites of fierce competition which originated evolutionally the concept of infection. In terms of human health, biofilms can contain pathogens, and there they are much better protected against disinfectants and biocides. As a stress response, they can enter a viable-but-nonculturable (VBNC) state, in which they are alive but cannot be de-tected by standard cultivation methods. These are reasons for the potential of biofilms as persistent sources of contamination. Understanding the biofilm life style helps to live with biofilms rather than to try to eliminate them with doubtful success.

BTV3-FG
Catalytic Biofilms: Linking EPS Composition to Solvent Stress

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Biofilms are potent biocatalysts due to their robustness, their ability to self-immobilize and regenerate and thus laying the ground for continuous process design [1]. However, although biofilms are in general described to be more resistance against all sort of stress, there are no detailed studies really looking at the reaction of biofilm growing microbes towards industrial relevant stress factors. In this study we focus on the phenotypic response of biofilm growing cells towards organic solvents. Especially the formation of EPS (extra polymeric substances) is under investigation in this respect, as EPS is a main part of the stress related response mechanisms in Biofilms as shown for biofilms exposed to high salt concentrations or heavy metals. In this study we analyze the changes in the EPS composition as a response to perturbation experiments with process relevant solvents, as well as the changes in absolute amounts of EPS.

To access EPS formation rate and composition we established various microscopic and biochemical methods. Confocal microscopy combined with various fluorescent dyes specific for certain EPS compounds revealed that the absolute amount of EPS is boosted upon exposure of the biofilm toward styrene. Interestingly a detailed analysis of the EPS composition via chemical methods after EPS extraction could only partly confirm this observation. This is probably due to the tedious task of EPS extraction, which is known to be not quantitative. Either EPS extraction is not complete or cells are also lysed during the separation process, leading to a contamination of the EPS sample [2]. Furthermore, the chemical analysis revealed that EPS of Pseudomonas sp. VLB120 is mainly composed of polysaccharides, lipids and proteins. Extracellular DNA (eDNA) seems to play an essential role for initial attachment, while it more or less disappears in mature biofilms. The ratio of these compounds change upon addition of solvent. Most strikingly lipids are not detectable anymore in course of solvent stress and biofilm age.
This paper will present a comparison of EPS extraction methods, a detailed composition of the Pseudomonas sp VLB120 EPS and its response to various solvents.

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BTV4-FG
Microstructured bioreactor surfaces as supports for catalytic biofilms

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Biofilms are complex agglomerates of microorganisms and extracellular polymeric substances which are produced during surface-attached growth of the microorganisms. Whereas biofilms are applied since decades for wastewater or odor treatment on an industrial scale, their exploitation in biotechnological production processes is still limited. While the robustness of biofilms is a serious problem in many technical processes (e.g. fouling and blocking of heat-exchangers) this trait could be very useful concerning continuous production processes, where the organisms are immobilized in a biogenous matrix produced by themselves. The use of (single-species) biofilms within catalysis requires a comprehensive knowledge of biofilm growth as well as its maintenance and catalytic properties. These factors depend on the environmental conditions, the properties of the provided surface (substratum) for biofilm growth and the characteristics of the selected organism. Therefore it is necessary to provide a well suited substratum for biofilm growth and to adjust the optimal conditions to perform the desired (bio)transformation. In terms of establishing monospecies biofilms the corresponding biofilm support must be sterile and should be resistant to damage. Thus metallic materials are well suited as substratum for the microorganisms. Whereas it is known that the topography of a surface has significant influence on the settlement of microorganisms its impact on the catalytic activity of a biofilm is currently unknown.
Within the framework of the Collaborative Research Center 926 (title:"Microscale Morphology of Component Surfaces") the topography-related growth as well as catalytic activity of different microorganisms attached to microstructured metallic surfaces is investigated. The contribution gives an overview about this collaborative research and initial results of the project are presented. The required modification of the surface is done by micro-milling technology, which enables the generation of reproducible grooves within the dimension of several micrometers (> 10 μm). These microstructured metallic surfaces are exposed to model organisms in a novel perfusion reactor as well as in a stirred tank reactor. Structure elucidation of the generated biofilms is done by confocal laser scanning microscopy, while the matrix is analyzed by chemical means. These analytical techniques are accompanied by single cell force spectroscopy and dynamic contact angle analysis to investigate the initial adhesion of the microorganisms on non-modified and modified metallic surfaces. The experimental data shall allow the construction of novel structured components to provide supports for biofilm reactors which might improve growth and catalytic activity of biofilms.
This project is financially supported by the German Research Foundation (SFB 926/1-2013).

BTV5-FG

Biofilm growth inhibition on medical plastic materials using immobilized esterases and acylases

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Bacterial biofilms cause persistent human infections, which are often related to foreign materials being introduced into the human body, such as implants or catheters[1]. More than 80 % of all human infections are caused by biofilms and in the U.S. 550,000 people die every year because of biofilm infections which equals the number of cancer deaths[2]. One reason for this is that bacteria within a biofilm show up to 1000 fold improved antibiotic resistances compared to their planktonic lifestyle[3].
The development biofilms is mediated by cell-to-cell communication via small diffusible molecules. Enzymatic degradation of the signal molecules, referred to as quorum quenching (QQ), is a promising approach to control biofilm growth.
The aim of this project is to find and apply QQ-enzymes that can prevent biofilm formation on medicinal materials. Commercially available enzymes as well as enzymes derived from metagenomic libraries were screened for AHL hydrolytic activity, *in vivo* quorum quenching and biofilm growth inhibition. Discovered QQ-enzymes were covalently immobilized via glutardialdehyde linker and preceding amino-functionalization of the corresponding materials.
A tube flow assay was developed, providing the possibility to monitor biofilm growth and test preventing enzymes on different medicine related materials, such as silicone or PVC.
Pseudomonas aeruginosa biofilms were analyzed by crystal violet staining and fluorescence microscopy for enzymatic growth inhibition.
Enzymes were found which could inhibit biofilm formation between 50 - 90 % when immobilized on different surface materials. These results are being further investigated e.g. regarding CLSM time curve analysis and offer a very interesting approach for further development of medicinal material applications.
The work was carried out within the “ChemBiofilm” project, funded by the BMBF.

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BTV6-FG

Engineering biology: From biodiversity to “Designer Bugs”

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Industrial “white” biotechnology is regarded as a central feature of the sustainable economic future of modern industrialized societies and is seen as one key to the European industry’s future global competitiveness, combining efficiency, the use of renewable resources and environmental friendliness to produce high-value products. Highly effective enzymes, heavily engineered microorganisms and “designer bugs” promise improvement for existing processes or could enable novel product ideas, paving the way to a knowledge-based Bioeconomy [1; 2]
Both, the replacement of traditional processes for the production of industrial chemicals by multi-step biosynthetic processes and the establishment of novel products and corresponding sustainable processes are based on fairly recent technological innovations in fields such as microbial genomics, metagenomics [3; 4], biocatalysis, in-vitro and in-vivo evolution [5]. In the past decade, systems biology has revealed great insights into metabolic pathways and regulatory networks, extending the knowledge-basis for the rational construction and for the bio-inspired design of producer microorganisms. Great potential lies in the reduction of cellular complexity by orthogonalization of metabolic modules. Recent advances will enable metabolic engineers to predict, design, and build streamlined microbial cell factories with reduced time and effort [6].

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BTV7-FG

The microbial ecology of aerobic granular sludge

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Aerobic granular sludge is recent development in wastewater treatment allowing a reduction of 75 % in size of the treatment and 30 % saving in cost and energy need. The sludge granules consist of a complex microbial community conducting aerobic, denitrifying and anaerobic conversions. The presentation will discuss process design aspects related to microbial ecology and the different nature of granular versus flocculent activated sludge technology.

BTV8-FG

Applied respiration: What dissimilatory iron reducers and electrolithotrophs can offer applied sciences

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Microbes can respire on ferric iron. Behind this rather simple statement lies a number of fundamental novel findings in scientific areas ranging from physiology and biochemistry to geology. Ferric iron is under neutral pH conditions an insoluble electron acceptor. Hence, microbes had to find a way to evolve an extended respiratory chain to the cell surface to reach the insoluble iron particles. More recently it turned out that the final reductases of the organisms are rather unspecific and also recognize an anode (for example made out of cheap carbon based materials) as terminal electron acceptor. In other words these microbes are capable of producing electric energy while eliminating organic carbon. Certainly, this ability can be used in applied areas like waste or waste water treatment. Aeration or the addition of another electron acceptor that is used up by the organisms is not necessary. Anodes are stable electron acceptors that cannot be depleted. Besides simple carbon elimination other areas in applied sciences might benefit from this technology as well. In the last years it turned out that microbes cannot only produce electricity but that they can also thrive using an electric current as electron and energy source and carbon dioxide as electron acceptor. Hence, there are microbes that can catalyze the opposite reaction of dissimilatory iron (or anode) reducers. If we will be able to handle and genetically engineer these organisms, they could provide us with a way to start biotechnological production processes with the renewable energy source electricity and carbon dioxide. This talk will give an overview about the biology and biotechnology of microbes that can reduce an anode or catabolically use cathodic electrons.

BTV9-FG

Electrifying white biotechnology: Microbial bioelectrocatalysis & electrochemical steered fermentation

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Converting electric energy into chemical energy carriers and fine chemicals will be one core-requirement of a seminal bioeconomy. Thus, microbial bioelectrochemical systems (BES) that interface electrodes and living microbial cells for microbial bioelectrocatalysis and electrochemical steered fermentations can play a key-role. Commonly all BES originate from microbial fuel cell (MFC) technology, yet the number and diversity of potential application increases. Among these, the exploitation of BES for the production and upgrading of fine and platform chemicals is regarded as one of the most promising ones. Thereby, BES combine the metabolic versatility and flexibility of microorganisms with the utilization (or even gain) of electric energy for the production of chemical compounds. The presentation will span from a short introduction into the fundamentals of BES via the bioelectrocatalytic production of H2, and acetic acid as well as the steered glycerol fermentation to their future potential in White Biotechnology.

BTP1-FG

Axillary bacteriotherapy as a promising technique to treat bromhidrosis

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Fecal transplantation, also called bacteriotherapy, has been successfully applied in the human gut for the treatment of *Clostridium difficile* [1]. In this research, we report a successful bacteriotherapy of the axillary microbiota to treat bromhidrosis. Bromhidrosis is a condition in which offensive body odour emanates from the skin, caused by bacterial breakdown of sweat secretions. A case study was performed on a monozygotic male twin who did not co-habit, in which one twin had a significant body odour and the other did not, as determined by a trained odour panel. Molecular analysis on their axillary samples showed significant differences, in which one twin had mostly corynebacteria as dominant bacteria, while the other twin had mostly staphylococci as dominant species in the axillae. *Corynebacterium* spp. were linked to the generation of body odour as they possess the enzymatic capacity to convert long-chain fatty acids to typical odorous short-chain fatty acids, while *Staphylococcus* spp. do not possess these enzymes [2]. Axillary bacteriotherapy was applied on one axilla of the odorous twin, using an axillary sample of the non-odorous twin. The other axilla of the odorous twin was not treated and used as a reference. Significant differences were seen for the hedonic value, the intensity and several odour characteristics on a short time scale after the treatment. Research is on-going to determine whether the enhanced odour profile is consistent. Axillary bacteriotherapy seems a promising technique to treat bromhidrosis.

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[3] We kindly acknowledge the cooperation of the twin and the odour panel.

BTP2-FG

Enzymatic Synthesis of Modified Purine Nucleosides by Thermostable Nucleoside Phosphorylases

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Nucleoside phosphorylases are attractive enzymes because they can catalyze the reversible phosphorolysis of purine or pyrimidine nucleosides effectively. Hence the enzymatic transglycosylations of nucleosides can be performed in a one-pot reaction by the simultaneous activity of pyrimidine nucleoside phosphorylase (PyNP) and purine nucleoside phosphorylase (PNP) [1]. However, low thermal stability and too strict substrate specificity of the enzymes are bottlenecks of the application. To overcome such limitations, we studied a set of thermostable nucleoside phosphorylases isolated from thermophilic microorganisms [2,3]: *Deinococcus geothermalis* (Dg), *Geobacillus thermoglucosidasius* (Gt), *Thermus thermophilus* (Tt) and *Aeropyrum pernix* (Ap). The enzymes in the present study including two PyNPs (TtPyNP, GtPyNP) and three PNPs (DgPNP, GtPNP and ApMTAP) were expressed in *E. coli* and characterized by thermostability studies, kinetic analysis, and substrate specificities. Here we report on the character of the enzymes and the results of the enzymatic synthesis.
The purified enzymes had temperature optima at 55°C (DgPNP), 60°C (GtPyNP) and 70°C (GtPNP). For TtPyNP and ApMTAP, their reaction rates kept increasing up to the water boiling point. The enzymes in the present study were incubated at their optimal temperatures (80°C for TtPyNP, 90°C for ApMTAP) for more than 8 h without significant activity loss. Natural substrates were effectively phosphorolyzed (activity level within 50-500 U mg^{-1}) by all investigated enzymes, 2'-modified (amino or fluro) nucleosides as well as 2(or 6)-halogenated purine base (for PNP) can be also accepted as substrates but at different activity levels.
By coupling using PyNP and PNP, the purine nucleosides were produced from a pyrimidine nucleoside which could offer a modified sugar moiety as a pentofuranosyl donor and a modified purine base which served as a pentofuranosyl acceptor. So far, 2,6-dihalogenated (Cl or F) ribosides or deoxyribosides are synthesized in high yield in 30 min (60-80%); 2-deoxyfluoro adenine ribo- and arabino- nucleosides were also successfully synthesized, however, with a moderate yield of 24% in 24 h.
We propose that the investigated enzymes are promising biocatalysts for the synthesis of the respective nucleoside analogues.

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BTP3-FG

The mechanistic role of a catalytic water molecule in discriminating between an aldolase and a transaldolase

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Transaldolases (Tal) catalyse the transfer of a dihydroxyacetone (DHA) moiety from a ketose donor e.g. fructose-6-phosphate (F6P) to an aldose acceptor e.g. erythrose 4-phosphate. This reaction proceeds via a Schiff base intermediate [1]. The fructose-6-phosphate aldolase A (FsaA) of *E. coli* is related to the Tal enzyme family [2]. It catalyses C-C bond formation between a ketose donor and an aldose acceptor via
Schiff base creating one product [3]. In a previous study, a DHA-dependent aldolase ($TalB^{F178Y}$) was engineered with a catalytic efficiency for the synthesis of F6P similar to $FsaA^{wt}$. $TalB^{F178Y}$ still retains Tal activity [4]. In FsaA and $TalB^{F178Y}$ a water molecule is coordinated by three side chains, whereas in TalB only two residues take part in the coordination [5,4,6]. Our research aims to completely convert a transaldolase to an aldolase and vice versa. Therefore, the coordination of the catalytic water molecule in the active site was investigated using site-directed mutagenesis. In FsaA, Gln^{59} and Tyr^{131}, two of the three residues which coordinate the catalytic water molecule were exchanged with the corresponding residues Glu^{96} and Phe^{178} in TalB and vice versa. The muteins $FsaA^{Q59E}$, $FsaA^{Y131F}$, $FsaA^{Q59E\ Y131F}$, $TalB^{E96Q}$ and $TalB^{E96Q\ F178Y}$ were successfully generated and purified. No differences in the secondary structure could be detected via CD spectroscopy, but the Tmelt values differed up to 18° C compared to the wt. The coordination of a catalytic water molecule in TalB by the same residues as in FsaA results in an aldolase activity but the reciprocal exchange in FsaA does not result in a transaldolase activity. Compared to $TalB^{F178Y}$, $TalB^{E96Q\ F178Y}$ showed a further reduction in Tal activity and increase in FsaA activity for the formation a F6P (improvement of V_{max} by 52%) and the cleavage reaction (doubling of V_{max}). A new mechanism for the formation of the Schiff base intermediate in FsaA will be proposed.

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EMV1-FG

Interspecies electron transfer in lake sediments

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Lake sediments are characterized by a more or less defined structural organization which determines the spatial order of microbially catalyzed redox reactions. Oxygen, nitrate, manganese, iron, sulfate and CO_2 are reduced in a typical sequence which follows the redox potentials of the associated reduction reactions and determines the available energy spans for the microbes involved. Also the biochemistry of key reactions in the degradation of comparably stable compounds differs between e.g. nitrate-reducing bacteria and strict anaerobes. Redox intermediates (hydrogen, formate, acetate, sulfur compounds) as well as partially degraded complex organics (humic compounds) mediate electron transfer processes between the various metabolic groups of bacteria active in these redox processes. Interspecies electron transfer via hydrogen or formate is essential in the degradation of fermentation intermediates by secondary fermentation processes to methane as final product. The energy available to the respective partners in these cooperations are at the minimum level that can barely support ATP synthesis and microbial life. Recent evidence has shown that also the degradation of easily fermentable substrates such as sugars can depend on interspecies electron transfer.

EMV2-FG

Microbial batteries in the seafloor: sulphide oxidation via long-distance electron transport

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Recently, an entirely novel type of microbial metabolism has been described from marine sediments, whereby filamentous bacteria are transporting electrons over centimeter-scale distances. By establishing such electrical circuitry, these micro-organisms are able to exploit spatially segregated pools of electron acceptors and donors, equipping them with a competitive advantage. First observed in laboratory experiments, we show that this novel electrogenic form of sulfide oxidation also occurs naturally in the seafloor. We observed the geochemical fingerprint of electrogenic sulphide oxidation at three coastal sites in the North Sea. These field observations reveal that the process occurs at sites with high rates of sulphide generation, and low rates of mechanical disturbance by infauna (faunal reworking destroys the electrical connectivity between sediment horizons). Overall, our observations indicate that electrogenic sulfide oxidation can strongly affect the biogeochemical cycling in a wide range of marine sediments, including coastal eutrophic systems with seasonal hypoxia, intertidal salt marshes, and possibly hydrothermal vent environments. The sensitivity towards faunal reworking constrains the distribution in the present ocean floor, but suggests a more widespread prevalence in geological times, before the rise of animal life.

EMV3-FG

Humic substance reduction and electron transfer to Fe(III) minerals under environmental conditions

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Humic substances (HS) are degradation products of biomolecules such as lignin, carbohydrates, lipids and proteins, and are ubiquitous in soils, sediments and waters at concentrations of up to 5-10 g C/g and 10-30 mg C/L, respectively. They contain redox-active functional groups such as quinones and have been shown to shuttle electrons between microorganisms and poorly soluble electron acceptors such as Fe(III) minerals [1]. Since HS can be reduced by a variety of microorganisms including Fe(III)-reducing, sulphate-reducing, dechlorinating and even methanogenic bacteria [1,2], electron transfer via HS has the potential to contribute significantly to electron fluxes in biogeochemical cycles in many environments.
However, although HS electron shuttling has been shown to significantly increase Fe(III) reduction rates in batch experiments, it is currently unknown, if and to which extent this process takes place in natural systems. This is due to the fact that most studies on HS electron shuttling have been carried out under laboratory conditions with artificial experimental systems of commercially available minerals [3,4] and humic substances or even HS analogues such as AQDS [4,5], well characterized pure bacterial cultures and under geochemical conditions which were often not consistent with environmental conditions such as very high concentrations of electron shuttles [1].In this presentation I will therefore present new data from experiments where we studied the process of HS electron shuttling between microbes and Fe(III) minerals under more environmentally relevant conditions. In particular I will present data on the mechanism and thermodynamics of electron shuttling and discuss the importance of electron shuttling in groundwater sediments.

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EMV4-FG

Hunting for microbial generators of electric currents in marine sediments

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Electric currents were recently reported to couple redox reactions in spatially separated layers of marine sediment (Nielsen et al., 2010). During this process, electrons for oxygen reduction are provided by the spatially separated sulfide oxidation. Long filamentous bacteria related to the deltaproteobacterial family *Desulfobulbaceae* were identified to mediate this centimeter-long distance electron transport (LDET) (Pfeffer et al 2012). To follow the development of electric currents and the growth of involved filaments *Desulfobulbaceae* we performed time series experiments. Microsensor measurements of O_2, pH, and ΣH_2S proved that long distance electron transport was well established after 10 days, reaching a current density maximum after 13 days. The detected depth integrated filament abundances over time implies an exponential growth with cell division distributed all along the multicellular filament. Analysis of single filament diameters showed different sizes at different time points but shared sequence identity of >98%. After 53 days of incubation the abundance of the filaments significantly decreased indicating that they experienced growth limitation. Although the LDET filaments showed a short life time in our incubation experiments, we were able to detect them in incubated sediments from the Little Sippewissett salt march (MA, USA) as well as *in situ* in sediments from the Lake Grevelingen (Holland) and in the Tokyo Bay (Japan). Their wide distribution indicates that they are worldwide present and our incubation experiments showed that they rapidly developed and dominated the biogeochemical processes, but just for a limited time.

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EMV5-FG

Chemotrophy associated with microbial sulphide oxidation by long-distance electron transport in coastal sediment

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Recently it was shown that filamentous bacteria transport electrons over centimetre-long distances in sulfidic sediments [1,2]. These bacteria belong to the Desulfobulbacea (DSB) and form dense filamentous networks that couple the sulphide oxidation at centimetre depth to oxygen consumption in surface sediments. These bacteria obtain their energy for growth from the oxidation of sulphide, but it is not known whether they are heterotrophic or chemoautotrophic. In this study we successfully induced the development of DSB filaments in coastal sulfidic sediments. Their presence was initially documented by their geochemical fingerprint, and later confirmed via FISH and cDNA clone libraries. Chemoautotrophy measured through 13C PLFA-SIP was strongly associated to the development of the filamentous networks. The peak in chemotrophic activity coincided with the deepening of the sulfidic zone in time. cDNA clone libraries also showed a large contribution of chemoautotrophic sulphur oxidizers belonging to the epsilon-proteobacteria (EPS). Community fingerprinting with DGGE confirmed the simultaneous development of EPS and DSB temporally and spatially in the sediment. Further examination of the PLFA signatures for active chemotrophs indicated that the EPS rather than the DSB were probably the main chemotrophic community.

However, we cannot discard the possibility of the DSB also contributing to chemotrophy since their PLFA pattern may be different from cultured relatives. In conclusion we propose a novel consortium of DSB filaments and EPS that regulate the oxidation of sulphide via a two-step process in coastal sulfidic sediments.

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EMV6-FG

Key factors in syntrophy: Genome comparison between the syntroph *Pelotomaculum thermopropionicum* and the non-syntroph *Desulfotomaculum kuznetsovii*

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Syntrophy is important in the degradation of organic compounds in methanogenic environments. Syntrophic conversion of organic compounds is driven by interspecies hydrogen and formate transfer. Hydrogen transfer is best studied, but knowledge about formate transfer is increasing. Bacteria that grow in syntrophy with methanogens can be found within the Gram-negative and Gram-positive bacteria. Phylogenetically, such bacteria are often similar to bacteria that cannot grow syntrophically. However, it is not completely understood which properties determine the ability to grow syntrophically or not. *Pelotomaculum thermopropionicum* and *Desulfotomaculum kuznetsovii* are a good example of phylogenetically closely related bacterial strains that differ in syntrophic capacity. Both strains belong to *Desulfotomaculum* cluster 1. *D. kuznetsovii* is the closest non-syntrophic strain related to *P. thermopropionicum* of which the complete genome is available. *P. thermopropionicum* is known for its ability to grow with propionate and ethanol in syntrophic association with methanogens, while *D. kuznetsovii* cannot grow syntrophically, but couples the degradation of these compounds to sulfate reduction. Additionally, *P. thermopropionicum* is not able to grow with sulfate as electron acceptor. Therefore, by comparing the genome of these strains, differences are expected in genes related to syntrophic growth and sulfate reduction. Genome comparison revealed that the genes involved in propionate and ethanol degradation pathways are highly similar. It also revealed that *P. thermopropionicum* possesses membrane associated formate dehydrogenases and hydrogenases, while *D. kuznetsovii* lacks such membrane associated enzymes, suggesting their important role in syntrophy. On the other hand, *P. thermopropionicum* lacks the adenylylsulfate reductase beta subunit, likely causing its inability to use sulfate as electron acceptor.

EMV7-FG

Long distance electron transfer in groundwater

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Spatially separated biogeochemical reactions are coupled by electric currents in marine sediments [1]. These electric currents are generated by multicellular "cable" bacteria, which are capable of a long-range electron transport from the lower sulphidic zone to the upper oxic zone. Thereby, they are bridging a suboxic zone over centimetre distances, where neither sulphide nor oxygen is present. Thus, electrons gained from sulphide oxidation are reducing dissolved oxygen to water in the oxic zone several mm away. These spatially separated redox half-reactions are exhibiting a characteristic pH profile of elevated pH in the oxic zone due to proton consumption and an acidification below the suboxic zone due to sulphate generation.
The observed cablebacteria are a new member of the family *Desulfobulbaceae* within the Deltaproteobacteria [2]. Since contaminated groundwater aquifers often showed a high abundance of *Desulfobulbaceae*, we searched for long-range electron transport in these freshwater environments. Therefore, we performed column experiments with sediment from the Düsseldorf Flingern aquifer. The homogenized sediment was incubated in the dark under pristine conditions and after adding different amounts of FeS, serving as electron donor and visual redox indicator. We measured depth profiles of the pore water chemistry in regular time intervals.
After two weeks of incubation a reproducible pH peak (up to pH 8.4) was measured in the oxic zone, indicating proton consumption due to oxygen reduction. Below the oxic zone, a local pH minimum occurred probably due to sulphate generation. Visual observations revealed first indications of a suboxic zone after one month of incubation, but further investigations of the pore water chemistry and the microbial abundance are required in order to prove the existence of long-range electron transfer in groundwater environments.

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EMP1-FG

Effect of energy flow on the susceptibility of aerobic methanotrophic communities to disturbance

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At the surface of flooded soils and sediments, methanotrophic bacteria thrive on the oxidation of methane. Methane is supplied from the anoxic bulk soil below, and oxygen from the overlying water body. The spatial separation of the two substrate sources leads to the formation of overlapping gradients of methane and oxygen, with minima where the methanotrophs are active. Irrespective of methane source strength, the substrate concentrations at the interface are comparably low. Only the location of the oxic-anoxic interface and, hence, the flux at the interface is affected. The energy flow as a potential factor affecting the methanotroph community was neglected so far. Here we demonstrate that the energy flow is influencing methanotrophic community's structure as well as population dynamics.
By diluting a native into a g-ray sterilized soil (1:40) we simulated a severe die-off of the microbial community, including methanotrophs. This disturbance allowed us to follow the re-establishment of a methanotroph community as a function of energy flow. Community structure was analyzed by T-RFLP, a diagnostic microarray, and by competitive RT-PCR targeting the *pmoA* gene, a functional and phylogenetic marker for methanotrophs. *pmoA* transcripts served as a proxy for species-specific activity. In general, *Methylobacter* related methanotrophs (type I) recovered faster under high energy flow. *Methylocystis* and *Methylosinus* methanotrophs (type II) were not significantly affected by the energy flow, but rather by disturbance in general. Hence, higher energy flows seem to select for a more resilient type I dominated community.
However, in the undisturbed control incubations, we observed a shift from type I to type II methanotrophs under high energy flows. We hypothesize this to be the result of a higher resistance of type II methanotrophs to grazing. This is consistent with earlier experiments on the susceptibility of methanotrophs to grazing, where type I methanotrophs were shown to be stronger affected by protozoan grazers.

EMP2-FG

NO-reductase-like enzymes - diversity and possible functions

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In recent years the known diversity of hydrocarbon activation mechanisms under anaerobic conditions has been extended by intra-aerobic denitrification. For two unrelated bacterial species, the NC10 phylum bacterium *Methylomirabilis oxyfera* [1] and the γ-proteobacterial strain HdN1 [2] it has been shown that, under anoxic conditions with nitrate and/or nitrite, mono-oxygenases are used for methane and hexadecane oxidation, respectively. No degradation was observed with nitrous oxide (N_2O) only. In the anaerobic methanotroph *M. oxyfera*, which lacks apparent nitrous oxide reductase in its genome, substrate activation in the presence of nitrite was directly associated with both O_2 and N_2 formation. These findings strongly argue for the role of nitric oxide (NO), or an oxygen species derived from it, in the activation reaction of methane. Although intracellular oxygen generation has been experimentally documented and elegantly explains the utilization of 'aerobic' pathways under anoxic conditions, research about the underlying molecular mechanism has just started. The proposed candidate enzymes for oxygen production from NO, an NO dismutase (NOD) [3], related to quinol-dependent NO reductases (qNORs), is present and highly

expressed in both *M. oxyfera* and strain HdN1. Besides that, several recently sequenced species from the *Cytophaga-Flavobacterium-Bacteroides* group harbor Nod/Nor genes, but experimential evidence is needed to show if these have NOD activity, are unusual but functional qNORs, or represent transition states between the two. This question is being addressed by a combination of sequence comparison, physiological experiments and expression studies in order to obtain an understanding of the function of these intriguing enzymes.

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SYV1-FG
Characterizing *Neisseria* species using ribosomal multilocus sequence typing

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Bacterial species have been defined using a range of phenotypic and biochemical approaches, DNA-DNA hybridization, and phylogenetic relationships employing *16S* and *23S rDNA* gene sequences. Recently, however, species delineation using multiple protein-coding gene sequences [1] has become feasible as more whole genomes are sequenced.
Ribosomal Multi Locus Sequence Typing (rMLST) has been proposed as a rapid characterization scheme that encompasses all levels of bacterial diversity, providing a rational and universal approach to bacterial classification based on the translation apparatus [2]. This approach indexes variation in the 53 genes encoding the bacterial ribosome protein subunits (*rps* genes). The *rps* loci are ideal targets for a bacterial characterization scheme as they are universally present, distributed around the chromosome, and encode conserved proteins. Collectively, the *rps* loci reveal variation that resolves bacteria into groups providing significantly more resolution than *16S* or *23S rDNA* gene phylogenies.
Recently, this approach has been employed to investigate taxonomic relationships within the genus *Neisseria* [3]. The groupings were not entirely congruent with current species designations, suggesting that some changes in nomenclature are required. These data showed that isolates classified as *Neisseria polysaccharea* include more than one taxonomically distinct organism. Further analyses using rMLST have shown that a species recently described as *Neisseria oralis sp. nov.* [4], is the same species as "*Neisseria mucosa var. heidelbergensis"*, described in 1971 [5].
Genome sequences from representative *Neisseria* strains are available from the curated database: http://pubmlst.org/neisseria/ [6], which allows rapid and computationally non-intensive identification of the phylogenetic positions of *Neisseria* species within the genus.

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SYV2-FG
Importance of MLSA for classification of representatives of the genus *Avibacterium*

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The aims of this investigation were to analyse members of *Avibacterium* by MLSA (multilocus sequence analysis) and MLST (multilocus sequence typing) in order to improve and clarify classification including a number of unclassified isolates (1). Members of the genus *Avibacterium* have been isolated from birds in which they probably occur as opportunistic pathogens, with the exception of *Av. paragallinarum* which is considered a primary pathogen (2). *Avibacterium* was formed by reclassification of the type species *Pasteurella gallinarum* and the taxa *P. avium*, *P. volantium* and *Pasteurella* sp. A and [*Haemophilus*] *paragallinarum* to *Avibacterium* (2). *Av. endocarditidis* was recently reported. Partial sequences were analysed for the five genes *rpoB*, *pgi*, *recN*, *sodA* and *infB* from the 49 isolates in addition to reference strains (1). Comparison between phylogenies from the five genes showed high divergence. Only three groups defined by bootstrap values higher then 50 % were found in the majority of trees. For bacterial species, gene phylogenies might be incongruent for isolates belonging to the same species in cases of a panmictic population structure, but they should be congruent among different species (3). The close relationship and high similarity between isolates included, except for the group with *Av. paragallinarum*, could support that some members of *Avibacterium* are incipient species. An incipient species has not fully diverged to become a new species, and still has some traits from another species. A minimum spanning tree supported the theory of incipient species since all isolates analysed shared at least one allele (identical sequence). However a narrow relationships was found by pair-wise comparisons of the whole genomic sequences of type strains of *Av. gallinarum Av. paragallinarum* and *Av. endocarditidis* which resulted in ANI (average nucleotide identity) values of between 83 and 95 %. These values were converted to from 35 to 70 % DDN (DNA denaturation). The 35 % determined by ANI was in excellent correspondence to the 34 % DDN determined previously (4). Unfortunately, the DDN between *Av. avium* and *Av. volantium* type strains (4) could not be repeated with the same method of DNA-DNA hybridization and also for *Av. avium* and *Avibacterium* species A we did not find evidence that clearly supported separate genomospecies. The present investigation indicates that *Avibacterium* should be reclassified leaving only two or three species in the genus.

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SYV3-FG
New approaches to improve the bacterial species definition (or 'identification')

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Taxonomy is a scientific specialty that, as a final goal, produces a classification system of biological units that serves as a communication tool among scientists of different disciplines. The research activities in the taxonomy of prokaryotes have improved concurrently with technological developments, especially in molecular biology. For many years, microbiologists have recognised that the number of described microbial/bacterial species is much too small in comparison with the expected diversity thriving in the biosphere. There is general agreement that taxonomists need to speed up the process of classifying prokaryotes. However, it seems that, at least, in western countries, the number of taxonomists is suffering a decline in promoting taxonomic expertise [1]. Contrarily, there is a significant increase in taxonomic activities in eastern countries, which has been responsible for most of the current growth in new microbial descriptions in taxonomic journals. Unfortunately, most of the new classifications are based on single isolates and often are restricted to the minimal standards guaranteeing the publication. Both of these recent practices create significant problems for the stability and operationality of the classification system. The new '-omic' technologies offer important advantages also for taxonomy [2]. The generation of extensive databases that can be used interactively can help in modernizing taxonomy. In this regard, genetic tools, such as the LTP [3] and JSpecies [4] can improve and enhance the species descriptions at the genotypic level. However, phenotypic-based methods, such as MALDI-TOF Mass Spectrometry of whole cell profiles [5] and metabolic profiling by means of high-resolution mass spectrometry [6] seem to be promising tools for classification and identification.

How to shift to a modern taxonomy, based on new tools for defining species and higher taxa will be discussed.

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MCV1-FG
Compartmentalization in *Planctomycetes* - Structure and function of cell complexity in bacteria

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Members of the phylum *Planctomycetes* within the domain Bacteria are distinguished by the complex organization of their cells, in which internal membranes separate the cell into major compartments(1, 2). In all planctomycetes examined, an intracytoplasmic membrane divides the cell into a ribosome-free outer region from ribosome-containing remainder of the cell, and in some planctomycetes these are the only two major compartments. In the clade of strains including *Gemmata obscuriglobus*, a third compartment analogous to a eukaryote nucleus occurs in which the nucleoid DNA is surrounded by an envelope consisting of two closely apposed membranes. In the distinctive clade of anammox planctomycetes, a third compartment also occurs, the anammoxosome, surrounded by only a single membrane and containing the biochemical apparatus of anaerobic ammonium oxidation. We will consider the structural and functional features which correlate with these cell plans, and discuss the evolutionary and cell biology implications of such cell complexity for understanding how proto-eukaryotes and the eukaryote nucleus and endomembrane systems may have evolved.

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MCV2-FG
Three-dimensional reconstruction of bacteria with a complex endomembrane system

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Specialization of the cellular space by membrane-defined compartments has been one of the major transitions in the history of life. Such compartmentalization has been claimed to occur in members of the *Planctomycetes, Verrucomicrobiae* and *Chlamydiae* bacterial superphylum. We have investigated the three-dimensional organization of the complex endomembrane system in the bacteria *Gemmata obscuriglobus*. We reveal that the *G. obscuriglobus* cells are not 'compartmentalized' nor 'nucleated' and that their membrane organization is not different from, but an extension of, the 'classical' Gram negative bacterial one. Our results have implications for our definition and understanding of bacterial cell organization, the genesis of complex structure, and the origin of the eukaryotic endomembrane system.

MCV3-FG
Ignicoccus: physiology, complexity and compartmentalization in a hyperthermophilic crenarchaeon

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The hyperthermophilic crenarchaeal genus *Ignicoccus* comprises three species so far, *I. islandicus*, *I. pacificus*, and *I. hospitalis*. The latter species is also known for its ability to form a unique, archaeal biocoenosis with *Nanoarchaeum equitans*, the smallest known archaeon described today. Cells of *Ignicoccus* are strict anaerobes, gaining energy by H_2-S^0 chemolithoautotrophy. Both organisms are cultivated in the lab in a stable coculture. While *I. hospitalis* is able to thrive alone without any reservations, growth of *N. equitans* strongly depends on the adherence to *I. hospitalis* cells; *N. equitans* was shown to take up lipids, amino acids and probably nucleotides from its host.
Ignicoccus cells have several unusual physiological and structural features. For CO_2 fixation, they use the dicarboxylate/4-hydroxybutyrate pathway. They also grow as adherent cells, using extracellular fibres which are distinct from archaeal flagella. Cells exhibit a unique cell architecture and cell envelope structure, consisting of two membranes, but lacking an S-layer. The presence of two membranes results in the formation of two compartments: the inner one is tightly packed and contains amongst others the DNA, RNA, ribosomes, a cytoskeleton, and many enzymes; the intermembrane compartment (IMC) is enclosed by the two membranes, it is weakly stained only, i.e. its content of proteins and nucleic acids is comparatively low. It contains, however, numerous round or elongated, membrane-coated vesicles.
In our report, we will present our current data and models on the subcellular distribution of a variety of proteins, e.g. enzymes for generation of a H^+ gradient, biosynthesis of ATP and activated CoA, by immuno-labelling in 2D and in 3D; the results let us conclude that the "outer cellular membrane" is energized and that the IMC is the compartment for ATP generation. Thus, energy conservation is separated from information processing and protein biosynthesis in *Ignicoccus*. By employing various tomographic methods, we have generated 3D models of these cells, underlining their high structural complexity, despite its small genome. The putative evolutionary impact is discussed.

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MCV4-FG
Concept of compartmentation (not only) in microbes

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Compartmentation has long been regarded as a privileged characteristic of eukaryotic cells. Classical, membrane-defined compartments that are easily discernible in electron micrographs such as the nucleus, mitochondria, plastids, the ER, the Golgi apparatus and vacuoles are generally associated with cells of higher organisms whereas prokaryotes appear to be poorly structured and consist of "one single compartment only" as modern textbooks of cell biology state [1, 2]. Superficially seen, the important function of compartmentation, i.e. the separation of incompatible biological functions and allocation of protected space for certain biochemical reactions and/or physiological conditions, appears not to be of relevance for prokaryotes. But this view were deceptive for the situation in microbes for two reasons.
Firstly, eukaryotic cells are usually compared with *E. coli*, implicitly taking this model organism as a representative for all prokaryotes, which is misleading. Secondly, structural compartments that separate biochemical reactions from each other are not restricted to organelles that are bounded by lipid membranes.
Based on the functional aspect of compartmentation as discussed previously [e.g. 3, 4], we can regard corresponding mechanisms and structural solutions both in microbes and eukaryotes more comprehensively. Compartmentation occurs on various levels of complexity from macromolecular structures to membranous organelles in eukaryotic *and* prokaryotic cells. However, not all types of compartments are found in all microbial species.

This contribution will give an overview of strategies of effective compartmentation in microbes and will discuss a concept that allows us to compare and appraise the structural solutions observed in pro- and eukaryotes.

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MPV1-FG
Identification of a novel host-specific IgM protease in *Streptococcus suis*

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Streptococcus (*S*.) *suis* is an important pathogen, that causes meningitis, arthritis, serositis and other pathologies in pigs and which has the ability to cause serious infections in humans. *In silico* analysis showed that the *S. suis* genome encodes a protein with high homology to the well characterized IgG endopeptidase of *S. pyogenes* IdeS (or Mac1) [1, 2].
The objective of this study was to identify the function of this *S. suis* protein, designated IdeSsuis.
The results of this study showed that IdeSsuis degrades immunoglobulins (Ig) of the isotype M, but not IgG or IgA. IdeSsuis was found to be host-specific as it exclusively cleaves porcine IgM but not IgM from six other species including a closely related member of the *Suidae* family. As demonstrated by flow cytometry and immunofluorescence microscopy IdeSsuis activity modulates binding of IgM to the bacterial surface. Furthermore the isogenic *ideSsuis* deletion mutant is significantly attenuated in survival in the blood of a piglet immunized once with a homologous bacterin [3].
IdeSsuis is the first prokaryotic IgM-specific protease described indicating that this enzyme is involved in a so far unknown mechanism of host-pathogen interaction at an early stage of the host immune response. Importantly cleavage of porcine IgM by IdeSsuis is the first identified phenotype reflecting functional adaptation of *S. suis* to pigs as the main host.

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MPV2-FG
High resolution real-time imaging reveals a dynamic phosphoinositide pattern on the *Legionella*- containing vacuole

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The opportunistic pathogen *Legionella pneumophila* employs a conserved mechanism to replicate in amoebae and macrophages within a unique compartment called the "*Legionella*-containing vacuole" (LCV). Formation of LCVs requires the bacterial Icm/Dot type IV secretion system which translocates more than 250 "effector proteins" into the target host cell. For several years it has been known that *L. pneumophila* effectors SidM and SidC anchor to the phosphoinositide (PI) lipid PI(4)*P* on the cytosolic face of LCVs, where they interfere with host cell vesicle trafficking and signal transduction [1]. SidM and SidC are just two examples of numerous effectors which interact with PI lipids. PI-binding *L. pneumophila* effectors in turn present themselves as useful tools to analyze PI patterns of eukaryotic cells [2]. Recently, we discovered an *L. pneumophila* PI phosphatase, termed LppA, which is translocated into the host via the Icm/Dot T4SS and preferentially hydrolyses poly-phosphorylated PIs to yield PI(4)*P*.
Modulation of the *L. pneumophila* LCV PI pattern, for example PI(4)*P* accumulation or PI(3)*P* dynamics, is an Icm/Dot dependent process. Yet, the temporal and spatial patterning of LCV PI lipids during infection remains largely uncharacterized.
Recent microscopic approaches to real-time imaging of PI lipids in *L. pneumophila* infection in the genetically tractable social amoeba *Dictyostelium discoideum* have provided a high resolution picture of the dynamic PI pattern on the LCV.

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MPV3-FG
Mannitol, a compatible solute synthesized by *Acinetobacter baylyi* in a two-step-mechanism including a salt-induced and salt-dependent mannitol-1-phosphate dehydrogenase

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Members of the genus *Acinetobacter* are non-motile ubiquitous Gram-negative bacteria that can be recovered from very different sources such as soil, water and clinical speciments. Persistence of *Acinetobacter* strains in their environments is due to their high metabolic diversity and their ability to cope with physicochemical changes, such as osmostress caused by high salinity or desiccation. In previous studies we reported that *A. baylyi*, a close relative of the opportunistic pathogen *A. baumannii* which exhibits high resistance to desiccation, can cope with osmostress by uptake and accumulation of the compatible solute glycine betaine (1).
Here we have adressed the question whether *A. baylyi* is able to synthesize compatible solutes *de novo*. NMR analyses revealed that cells accumulated glutamate and the rather unusual sugar alcohol mannitol upon an increase of the external NaCl concentration. To unravel the pathway of mannitol biosynthesis, the genome was inspected for genes potentially involved in its biosynthesis. Two adjacent genes, a potential mannitol-1-phosphate dehydrogenase MtlD (ACIAD1672) and a potential mannitol-1-phosphatase (ACIAD1671) were identified. Expression of *mtlD* was highly induced at high salinity and the purified protein catalyzed the reduction of fructose-6-phosphate to mannitol-1-phosphate with a specific activity of 130 U/mg. The enzymatic activity was strictly salt dependent and deletion of *mtlD* resulted in a complete loss of salt-dependent mannitol biosynthesis. These data demonstrate that osmo-induced synthesis of mannitol is catalyzed by a two step pathway including a mannitol-1-phosphate dehydrogenase which mediates the first step of this pathway and is regulated by salinity on the transcriptional as well as on the activity level. Inspection of *A. baumannii* genomes unravelled a broad distribution of the *mtlD* gene suggesting a major role of mannitol in osmoadaptation of *A. baumannii* strains, which is currently under investigation.

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MPV4-FG
Campylobacter jejuni infection of infant mice: acute enterocolitis is followed by asymptomatic intestinal and extra-intestinal immune responses

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Background: *Campylobacter (C.) jejuni* is among the leading bacterial agents causing enterocolitis worldwide. Despite the high prevalence of *C. jejuni* infections and its significant medical and economical consequences, intestinal pathogenesis is poorly understood. This is mainly due to the lack of appropriate animal models. In the age of 3 months, adult mice display strong colonization resistance (CR) against *C. jejuni*. Previous studies underlined the substantial role of the murine intestinal microbiota in maintaining CR. Due to the fact that the host-specific gut flora establishes after weaning, we investigated CR against *C. jejuni* in 3-weeks-old mice and studied intestinal and extra-intestinal immunopathogenesis as well as age dependent differences of the murine colon microbiota.
Methodology/Principal Findings: In infant animals infected orally immediately after weaning *C. jejuni* strain B2 could stably colonize the

gastrointestinal tract for more than 100 days. Within six days following infection, infant mice developed acute enterocolitis as indicated by bloody diarrhea, colonic shortening and increased apoptotic cell numbers in the colon mucosa. Similar to human campylobacteriosis clinical disease manifestations were self-limited and disappeared within two weeks. Interestingly, long-term *C. jejuni* infection was accompanied by distinct intestinal immune and inflammatory responses as indicated by increased numbers of T- and B-lymphocytes, regulatory T-cells, neutrophils, as well as apoptotic cells in the colon mucosa. Strinkingly, *C. jejuni* infection also induced a pronounced influx of immune cells into extra-intestinal sites such as liver, lung and kidney. Furthermore, *C. jejuni* susceptible weaned mice harbored a different microbiota as compared to resistant adult animals.
Conclusions/Significance: These results support the essential role of the microflora composition in CR against *C. jejuni* and demonstrate that infant mouse models resemble *C. jejuni* mediated immunopathogenesis including the characteristic self-limited enterocolitis in human campylobacteriosis. Furthermore, potential clinical and immunological sequelae of chronic *C. jejuni* carriers in humans can be further elucidated by investigation of long-term infected infant mice. The observed extra-intestinal disease manifestations might help to unravel the mechanisms causing complications such as reactive arthritis or Guillain-Barré-Syndrome.

MPV5-FG

Coxiella burnetii harbours several anti-apoptotic type IV secretion system substrates with distinct molecular activities

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Manipulation of host cell apoptosis is a virulence property shared by many intracellular pathogens to ensure productive replication. For the obligate intracellular pathogen *Coxiella burnetii* anti-apoptotic activity, which is depending on a functional type IV secretion system (T4SS), has been demonstrated. Here, we describe the T4SS effector proteins CaeA and CaeB (***C****oxiella burnetii* **a**nti-apoptotic **e**ffector) that inhibit the intrinsic apoptotic pathway.
CaeA displays a nuclear localization when ectopically expressed. This effector protein interferes with apoptosis-induction probably by up regulating the protein kinase B (Akt) and the IAP (inhibitor of apoptosis) protein survivin.
CaeB displays a mitochondrial localization when ectopically expressed. This very potent anti-apoptotic protein did not cause alteration in the protein level of apoptosis regulators, but rather acted downstream of Bax activation and mitochondrial trafficking. Thus, CaeB does not prevent recruitment of Bax to the mitochondria but inhibits mitochondria outer membrane permeabilisation (MOMP) and as a consequence caspase 9 and caspase 7 activation. These data suggest that CaeB inhibits apoptosis at the mitochondrial level.
Taken together, *C. burnetii* harbours at least three anti-apoptotic effector proteins (AnkG, CaeA and CaeB). Our data suggests that these effector proteins localizes differently within the cell and uses diverse mechanism(s) to prevent host cell apoptosis.

MPV6-FG

Subtilase SprP regulates virulence phenotypes in *Pseudomonas aeruginosa*

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The facultative anaerobic Gram-negative bacterium *Pseudomonas aeruginosa* is an opportunistic human pathogen that causes chronic and acute infections and is a major agent of morbidity and mortality in cystic fibrosis (CF) patients [1]. *P. aeruginosa* produces an impressive arsenal of virulence factors, among them flagella and type IV pili, exopolysaccharides, lipopolysaccharides, and several secreted factors including different hydrolytic enzymes [2]. The production of these virulence factors is regulated by at least three different *quorum sensing* systems [3].
Within the genome sequence of *P. aeruginosa*, we have identified the open reading frame *PA1242* which presumably encodes a subtilase belonging to the peptidase_S8 family. This enzyme, which we have named SprP, differs from typical subtilases by containing a 233 amino acid domain of unknown function (DUF) located between the signal peptide and the conserved S8 domain. Interestingly, this DUF domain appears to be present almost exclusively in strains of the genus *Pseudomonas* thus defining a discrete class of *Pseudomonas* proteins. The proteolytic activity of SprP was demonstrated using the serine protease substrate Suc-AAPF-pNA after *sprP* expression in *Escherichia coli*. A *P. aeruginosa sprP* negative mutant was constructed and its gene expression levels were compared to the wild-type strain using a genome microarray. The deletion of the *sprP* gene resulted in an altered expression pattern of 218 genes with 116 genes up-regulated and 102 genes down-regulated. These transcriptome data indicated that SprP affects transcription of pyoverdine biosynthesis genes as well as several genes involved in denitrification. The changes observed at the levels of transcription were confirmed by respective physiological studies with *P. aeruginosa*. The *sprP* negative mutant showed strong cell aggregation during cultivation, an almost fivefold enhanced biofilm formation in the crystal violet biofilm assay, and a fivefold higher pyoverdine concentration in the culture supernatant. Furthermore, the *P. aeruginosa sprP* negative mutant showed reduced growth after 6h cultivation under anaerobic conditions and agar plate assays illustrated a complete loss of motility. In conclusion, the newly characterized protease SprP belongs to the peptidase_S8 family and is involved in the regulation of several virulence-associated phenotypes of *P. aeruginosa*.

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MPV7-FG

Metabolic adaptation of *Staphylococcus aureus* to nasal colonization reveals new antimicrobial target

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Colonization of the human nose by Staphylococcus aureus in one third of the human population represents a major risk factor for invasive infections. The basis for adaptation of S. aureus to this specific habitat and reasons for the human predisposition to become colonized have remained largely unknown. Human nasal secretions were analysed by metabolomics and found to contain potential nutrients in rather low amounts. No significant differences were found between S. aureus carriers and non-carriers indicating that S. aureus carriage is not associated with individual differences in nutrient supply. Based on the metabolomics data a synthetic nasal medium (SNM3) was composed and S. aureus isolates were found to grow consistently and express key genes in SNM3 in a similar way as in the human nose indicating that SNM3 represents a suitable surrogate environment for in vitro simulation of nasal colonization. While most S. aureus grew well in SNM3 most of the tested coagulase negative staphylococci had major problems to multiply in SNM3 supporting the notion that CoNS are less well adapted to the nose and colonization preferentially human skin. Global gene expression analysis revealed that *S. aureus* growth in SNM3 depends heavily on de novo synthesis of methionine. Of note, a methionine-biosynthetic inhibitor exhibited antimicrobial activity against *S. aureus* grown in SNM3 but not in complex media and the target enzyme was strongly upregulated in human noses indicating that this pathway may include promising antimicrobial targets that have previously remained unrecognized. Hence, exploring environmental conditions facultative pathogens are exposed to during colonization can be useful for understanding niche adaptation and identifying targets for new antimicrobial strategies.

MPV8-FG

Adaptation of *Staphylococcus aureus* to human nasal secretions

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Staphylococcus aureus colonizes the anterior nare of approximately one third of the human population without causing any symptoms. Nasal carriage of *S. aureus* is an important source of nosocomial infections and might be a result of adaptation and selection of certain *S. aureus* strains to the nasal environment. In the present approach, we investigated the adaptation of *S. aureus* Newman to nasal secretions derived from twelve healthy volunteers among them six permanent carriers and six permanent non carriers of *S. aureus*. *S. aureus* isolates derived from the carriers were characterized by MLST and DNA-arrays. Using GC-MS, 43 different metabolites have been identified and quantified in the nasal secretions.
To profile the protein expression pattern of *S. aureus* during adaptation to nasal secretions, green fluorescence protein (GFP) expressing *S. aureus* Newman cells were incubated with the twelve nasal secretions for 1 and 24 hours, respectively. GFP positive bacteria were separated via FACS sorting and the intracellular and surface-associated proteome was analyzed by mass spectrometry and quantified by isotopic labeling. Altogether we identified 1381 proteins including 850 bacterial and 531 human proteins. 417 of the bacterial proteins have been quantified. Additionally 55 proteins were exclusively found in *S. aureus* after exposition to the nasal secretions. From these analyses we found evidence that in the nasal secretions, *S. aureus* copes with various nutrient limitations. Amino acids and fatty acids were used as carbon and energy source. Several transport systems employing siderophores and the Isd system for utilization of iron in hemoglobin were found to be *de novo* synthesized which suggests limited amounts of iron. Moreover, enzymes producing glycine betaine to protect the bacterial cell from osmotic lysis were exclusively synthesized in cells exposed to the nasal secretions. From the analysis of regulatory loci involved in virulence gene expression and some virulence factors we got evidence that proteins necessary for tissue adhesion and immune evasion such as SdrD, NWMN_1069, ClfA, DltA, IsdA and NWMN_2317 are preferentially expressed in cells grown in nasal secretions whereas toxins and exoenzymes are repressed.

QDV3-FG

The use of MALDI-TOF MS or (Q)RT- PCR of microbiological water analyses. A safety issue for the consumer

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In the Netherlands drinking water is transported in the distribution network without adding disinfectants. Drinking water can be produced out of groundwater or out of surface water after extensive purification. Surface water normally contains a lot of bacteria and chemicals which are prohibited in drinking water according to the Dutch Law. Therefore the surface water production plants have complex purification steps to remove inter alia pesticides, herbicides, pharmaceuticals and also pathogenic microorganisms. For the reason that there is no chlorine in the distribution network to kill unwanted bacteria or viruses it is important to make safe drinking water and to contain it safe in the distribution pipes. Frequently new pipes are introduced in the system and sadly enough old pipes sometimes break by accident causing unwanted leakages. In those cases there is a risk that pathogens can enter the distribution network and so can contaminate the drinking water. The drinking water is microbiological monitored if a leakage is corrected and the water can be used again. It takes a minimum of 18 hours before it is known or the water is safe to drink (free of pathogens). It is not always possible to shut down the drinking water for that long time, so it has to be delivered to the consumer with a boiling advice and a risk of contamination. It is very important to know as soon as possible which bacteria are present and to know if they are pathogenic. Normally water is monitored by culturing techniques which consumes a lot of time. Last decennium new molecular techniques are introduced at WLN. For direct analyses Quantitative Polymerase Chain Reaction (QPCR) is used for some bacteria (1) and the Reversed Transcriptase PCR (RT-PCR) is used for others. Not all molecular methods are accepted and according to the law the reference methods are still the culturing techniques and therefore a rapid and reliable confirmation of the grown bacteria is essential for the water companies.
WLN has introduced besides the colony-PCR the MALDI-TOF technique. In 2012 the technique is validated for E*scherichia coli*, *Enterococci*, *Pseudomonas aeruginosa*, *Clostridium perfringens* and *Legionella* according NEN-EN-ISO 16140 (2). About 500 samples and 70 reference strains are compared with the classical identification and PCR identification. Except for Legionella there was 100% agreement between the MALDI-TOF-confirmation and the "classical "confirmations.
For Legionella there was about 94% agreement due to the fact that not all species were present in the database.
The routine use of the Malditof as a part of the microbiological monitoring of water minimizes the risk of drinking contaminated drinking water by the consumer.

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QDV4-FG

Scary meat? - Occurrence of antibiotic resistant bacteria (MRSA, ESBL) on conventional and organic retail meat samples in the greater area of Villingen-Schwenningen, Germany

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Antibiotic resistant bacteria represent a threat for human health, particularly by causing severe nosocomial infections. The widespread and uncritical use of antibiotics in industrial livestock farming is a major factor leading to the selection of antibiotic resistant strains. At the beginning of 2012 a study [1] published by the German BUND (Bund für Umwelt und Naturschutz, i.e. Friends of the Earth Germany) caused a stir nationwide by showing that ten out of 20 investigated retail chicken meat samples contained ESBL- (Extended Spectrum of Beta-Lactamase) producing enterobacteria, and at least two of them also contained MRSA (Multi Resistant *Staphylococcus Aureus*).
In the study presented here, 28 fresh meat samples (14 chicken and 14 pig samples, respectively), stemming from conventionally (16) and organically (12) grown animals were obtained from supermarkets and discounters in the greater area of Villingen-Schwenningen (Baden-Württemberg, Germany). They were screened for the presence of MRSA and ESBL-producing bacteria by means of plating meat homogenates on appropriate selective media. In addition, the presence of ESBL-producing bacteria was confirmed by using the double disk approximation test and by identification of suspicious isolates using a MALDI-TOF system (Maldi Biotyper, Bruker). Moreover, the bacterial load of the samples was determined following ISO 21528-2:2005 and DIN 10164-1:1986 using VRBD agar plates.
The bacterial load of chicken meat samples was significantly higher compared to pig samples, probably because they partially contained parts of the chicken skin. In addition, organic meat samples showed higher germ counts than the conventional ones. In contrast to the BUND study [1], all investigated samples were found to be free of MRSA and ESBL-producing bacteria. A few bacteria were isolated on the ESBL-selective plates and mostly identified as *Serratia* spp., however, all failed the double disk approximation test. Clearly, more studies are needed to better understand the factors influencing the occurrence of antibiotic resistant bacteria along the meat production chain in general, and on retail meat in particular. Germ numbers of up to 40 x 10^6 per g of retail meat, however, underline the need for strict hygienic measures when raw meat is processed, including roasting it thoroughly before consumption.

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RSV1-FG

Bacterial phosphorylation networks: the systems biology perspective

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Reversible protein phosphorylation on histidine, aspartate, serine, threonine and tyrosine residues has been established as an important regulatory mechanism in bacteria. A growing number of studies employing mass spectrometry-based proteomics report large protein phosphorylation datasets, providing precise evidence for *in vivo* phosphorylation of key bacterial proteins (1). These results have stimulated a number of follow-up studies, focusing on structural, functional and physiological consequences of individual phosphorylation events (2, 3). However, isolated studies do not capture the connectivity present in the system. Protein phosphorylation pathways emerge as large and interconnected networks, involving mutually activating protein kinases, kinases acting as network nodes by phosphorylating different substrates, and cross-talk of phosphorylation with other post-translational modifications. The complexity of these networks clearly necessitates the use of systems biology approaches (4). We argue that the next challenge in the field will be the large-scale detection of protein kinase and phosphatase substrates and their integration into regulatory networks of the bacterial cell. Phosphoproteomics represents the basis for detection of phosphoproteins and phosphorylation sites (5), but it must be combined with interactomics, transcriptomics and classical bottom-up approaches in any credible attempt to build *in silico* phosphorylation networks. The integrated systems biology approach to charting phosphorylation networks will be illustrated by the case of *Bacillus subtilis*, the Firmicute model organism.

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RSV2-FG

Protein arginine phosphorylation in *Bacillus subtilis*

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Modification of proteins by phosphorylation is an important ubiquitous mechanism to influence their activity and conformation. Recently, arginine protein phosphorylation by the kinase McsB has been added to the better-known protein phosphorylations at serine, threonine, tyrosine, histidine or aspartate [1]. Currently the physiological significance and role of this new protein modification is under investigation and it appears that in *Bacillus subtilis* the combined activity of a protein arginine kinase and phosphatase allows a rapid and reversible regulation of protein activity and that protein arginine phosphorylation play a physiologically important and regulatory role in bacteria [2].
The chemical, thermodynamic and kinetic properties of protein arginine phosphorylation warrants a more detailed analysis to understand the general role and impact of such a protein modification on protein function and regulation.

1. J. Fuhrmann, A. Schmidt, S. Spiess, A. Lehner, K. Turgay, K. Mechtler, E. Charpentier, T. Clausen, Science **324** (2009), p. 1323.
2. A.K.W. Elsholz, K. Turgay, S. Michalik, B. Hessling, K. Gronau, D. Oertel, U. Mäder, Jörg Bernhardt, D. Becher, M. Hecker, and U. Gerth, PNAS **109** (2012), p. 7451

RSV3-FG

Acetyl phosphate is a potent regulator of bacterial protein acetylation

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Reversible N^{ε}-lysine (protein) acetylation of bacterial proteins is a previously unrecognized regulatory mechanism. Since the study of bacterial protein acetylation is in its infancy, many questions remain unanswered. The mechanisms responsible for most protein acetylations remain obscure, their effect on protein function remains unclear, and their global impact remains unquantified.
Earlier studies provided evidence for this post-translational modification on about two hundred proteins in *E. coli* and *S. enterica*. We now report that that the acetylated proteome ('acetylome') is much larger than previously reported. Our preliminary experiments using a novel label-free quantitative mass spectrometry analysis identified 1515 unique acetylation sites on 541 *E. coli* proteins that function in diverse cellular processes, including signal transduction, transcription, quorum sensing, biofilm formation and pathogenesis. Some acetylated proteins include the response regulator RcsB, the global transcription factor CRP, and RNA polymerase. If only a fraction of the detected acetylations exert a significant impact on their protein's function, then this post-translational modification would easily surpass phosphorylation as the primary regulatory mechanism.
The Pta-AckA pathway links the central metabolite acetyl-CoA to ATP generation. The pathway intermediate acetyl phosphate has been shown to donate its phosphoryl group to two-component response regulators, e.g. CpxR and RcsB. We now propose that acetyl phosphate is a potent regulator of bacterial protein acetylation. Anti-acetyllysine Western immunoblot and quantitative mass spectrometry analyses revealed hyperacetylation of mutants that accumulate acetyl phosphate relative to the WT parent and to cells that do not synthesize acetyl phosphate. However, mutants that do not synthesize acetyl phosphate still acetylate proteins, albeit at substantially reduced levels. Taken together, these results argue for two distinct classes of acetylation mechanisms: acetyl phosphate-dependent mechanisms and acetyl phosphate-independent mechanisms. Efforts are underway to identify and characterize both classes of mechanisms.

RSV4-FG

Protein *S*-bacillithiolation as reversible redox-switch mechanism in *Firmicutes* bacteria

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Bacillus und *Staphylococcus* species utilize as major redox buffer bacillithiol (BSH) to maintain the redox balance. Under oxidative stress conditions, BSH reacts with protein thiols to form mixed BSH protein disulfides, termed as S-bacillithiolations. Protein S-bacillithiolation protects active site Cys residues against irreversible oxidation and functions as redox switch to control the activity of redox sensing transcription factors [1]. Using redox proteomics and mass spectrometry, we identified eleven *S*-bacillithiolated proteins in *B. subtilis* after hypochlorite stress, including the OhrR repressor, the methionine synthases MetE, the inorganic pyrophosphatase PpaC, the phosphoglycerate dehydrogenase SerA, the chorismate mutase (AroA), the translation elongation factor EF-Tu (TufA), the IMP dehydrogenase (GuaB), the ferredoxin-$NADP^+$ oxidoreductase (YumC) and the bacilliredoxin (YphP). The methionine synthase MetE is the most abundant *S*-bacillithiolated protein in *Bacillus* species after NaOCl exposure. *S*-bacillithiolation of MetE causes methionine auxotrophy in NaOCl-treated cells. The redox-sensing OhrR repressor is inactivated by *S*-bacillithiolation after NaOCl stress resulting in up-regulation of the OhrA peroxiredoxin that confers resistance to NaOCl stress. The bacilliredoxin YphP is *S*-bacillithiolated at its active site Cys and was shown to function in the de-bacillithiolation process [1].
S-bacillithiolation is wide-spread among Firmicutes with 8 conserved and 29 unique *S*-bacillithiolated proteins identified in *B. subtilis, Bacillus amyloliquefaciens, Bacillus pumilus, Bacillus megaterium* and *Staphylococcus carnosus* [2]. The *S*-bacillithiolome contains mainly

biosynthetic enzymes for amino acids (methionine, cysteine, branched chain and aromatic amino acids), cofactors (thiamine), nucleotides (GTP); as well as translation factors, chaperones, redox and antioxidant protein. *S*-bacillithiolation is accompanied by an increased BSSB level and a decreased BSH/BSSB redox ratio.
Together our data support a major role of the BSH redox buffer in redox control and protection of conserved and essential proteins against irreversible oxidation by *S*-bacillithiolations in *Firmicutes* bacteria.

1. B. K. Chi, K. Gronau, U. Mäder, B. Hessling, D. Becher, and H. Antelmann *Mol Cell Proteomics* 10 (2011), M111.009506.
2. B. K. Chi, A. A. Roberts, T. T. Huyen, K. Bäsell, D. Becher, D. Albrecht, C. J. Hamilton and H. Antelmann *Antioxid Redox Signal*. (2012) doi:10.1089/ars.2012.4686.

SIV1-FG
Monogastric model animals - untangling the interplay of microbiota with the development of its mammalian host

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Soon after birth the gastrointestinal tract (GI) of humans, pigs and other monogastric animals is colonized by a myriad of microbes, generally referred to as GI tract microbiota. This microbiota plays an important role in the host's health and nutrition. This intricate interplay between microbial colonization in early life and the development of the mammalian host is addressed in the INTERPLAY project, an EU-FP7-funded collaborative project integrating the expertise of 11 partners from around and beyond Europe.
The GI tract microbiota is characterized by its wide phylogenetic and functional diversity. Despite all efforts in improving the cultivation of novel GI tract microbes, the use of culture-independent approaches is crucial to provide a comprehensive picture of the GI tract microbial functioning. Since the introduction of culture-independent approaches, mainly those based on 16S ribosomal RNA (rRNA) and its encoding gene, GI tract ecology has experienced a revival. These culture-independent approaches gave insight into the temporal, spatial and inter-individual microbial diversity in the GI tract of humans and animals. The past years major developments have been made in high throughput methodologies to characterize microbial communities. Novel technologies, such as barcoded pyrosequencing of 16S rRNA genes, as well as phylogenetic fingerprinting using DNA microarrays including the Human, Mouse, Pig and Poultry Intestinal Tract Chips have recently been described. Since multiple samples can be analyzed in detail in a rather short time, these approaches offer great potential in finding significant correlations between the GI tract microbiota compositional signatures and the health status of the host. In addition, several meta-omics studies have been developed, which allow studying the genetic potential and functional properties of the GI tract microbiota. The application of these approaches to understanding the interplay of intestinal microbiota and production animal health, also in response to the production environment as well as dietary additives, can provide the necessary knowledge for the development of innovative nutritional strategies towards more sustainable animal production. Examples will be provided from current research on the pig, and an overview of the current state of the art of microbiomics research will be given.

SIV2-FG
Systems approaches to understand the interplay between gut microbiota and its mammalian host

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Understanding complex microbial ecosystems such as those found in the mammalian intestinal tract requires a systems biology approach robust enough to take into account the enormous compositional and functional diversity of microorganisms inhabiting this environment, as well as the significant functional redundancy and inter-individual variation within the microbiota. Furthermore, the vast majority of bacterial species inhabiting the intestinal tract remain uncultured, which severely limits the investigation of their physiology, metabolic capabilities, and role in the environment. The microbiota of the adult intestine is stable, however, it has also been shown recently that the small intestinal microbiota is characterized by more dynamic temporal fluctuations in composition and activity, as was shown by 16S rRNA- and metatranscriptome-based studies. Currently, the most efficient way to study the metabolic interactions of microbiota in vivo is by applying high-throughput sequencing of RNA transcripts (RNA-seq) and metaproteomics for the comprehensive analysis of microbiota-wide gene expression data as proxy for protein production and ultimately (and up to a certain extant) as proxy for metabolic activity and fluxes. Metabolic flux analysis, as measured by isotopic labeling, can provide valuable information on the global metabolic profiles of the microbiota as a whole under the specific conditions.
Some of the current bottom-up efforts towards the elucidation of microbial interactions in microbiota include attempts for the genome-scale, constraint-based modeling of the metabolism of key players and the subsequent coupling of the individual networks for instance, by linking the exchange fluxes in microbial networks of a small and well-defined number of players. Nevertheless, the applicability as such for much larger and complex communities like the gut microbiota is - as yet - quite limited. Besides the fact that it is often not clear what are the key players and that these anyway change as a result of the dynamics of the feed/food-host interactions, from a genomic point of view, it becomes very difficult to make accurate predictions regarding the metabolic interactions - and dynamics- within the microbiota. Rather, through thorough bioinformatic analysis, multivariate statistics and reverse engineering, available data sets can be used to map and define the genomic and expression modules reflecting the actual metabolic functionalities within the microbiota, which can be subsequently tackled through constrained-based modeling.
I will give a short overview of these systems approaches to disentangle the interplay of function of gut microbiota with its mammalian host and will provide two relevant examples of this.

SIV3-FG
Obesity and the gut microbiota: the SIHUMI mouse model provides insights into relationships

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Background: Obesity has been attributed mainly to an unhealthy life style. However, the proportional change of Firmicutes and Bacteroidetes in obese mice and humans link the gut microbiota to obesity. Members of the Erysipelotrichaceae, a family within the phylum Firmicutes, increase in response to high fat feeding [1]. Species of this family are possible candidates for promoting obesity development and related pathologies. Several explanations for a microbial contribution to these diseases are discussed: metabolic endotoxemia, an efficient energy extraction from the diet or an increased gut permeability. The use of a gnotobiotic mouse model offers the opportunity to gain insight into the development of diseases in dependence of different microbial communities.
Methods: We established a mouse model with a simplified human intestinal microbiota (SIHUMI) consisting of eight bacterial species: *Anaerostipes caccae, Bacteroides thetaiotaomicron, Bifidobacterium longum, Blautia producta, Clostridium ramosum, Clostridium butyricum, Escherichia coli* and *Lactobacillus plantarum* [2]. The species *C. ramosum* is a member of the family Erysipelotrichaceae. The use of SIHUMI mice containing only seven bacterial species without *C. ramosum* (SIHUMI-Cra), allows us to clarify the effect of *C. ramosum* on the development of symptoms of the Metabolic Syndrome.
Results: SIHUMI mice fed a high fat diet (HFD) for four weeks gain body weight and body fat and stay lean on a low fat diet (LFD). SIHUMI-Cra mice fed the HFD gained significantly less body weight and body fat than SIHUMI mice fed the HFD. These mice did not differ in their energy intake or the energy they digested, suggesting a key role of *C. ramosum* in this observation. Gut permeability, formation of short chain fatty acids and cytokine expression levels were analysed in all mice to obtain hints on the underlying mechanisms.
Conclusions: Gnotobiotic mice deliver an opportunity to investigate the relationship between the host and the gut bacteria in the development of diseases. The data obtained from the SIHUMI mouse model emphasize the role of *C. ramosum* in obesity in this mouse model. Mice monoassociated with this bacterium permit us to identify its possible adipogenic factor.

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2. N. Becker, J. Kunath, G. Loh and M. Blaut, Gut Microbes 2 (2011), p. 25-33

SIV4-FG

Localization of two major phylogroups of *Faecalibacterium prausnitzii* in human fecal samples

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Introduction and Objectives: *Faecalibacterium prausnitzii* is one of the most abundant bacteria in the human large intestine belonging to the firmicutes phylum. It has been shown that there is a significant decrease in numbers of the *F. prausnitzii* in inflammatory bowel disease, especially in patients with Crohn's disease. *F. prausnitzii* is a fecomucus bacterium, which means it colonizes the feces/mucus interphase as well as the luminal part[1]. Recently it was demonstrated that there are two major phylogroups of *F. prausnitzii*: phylogroup 1: with ATCC-27768 as the representative strain and phylogroup 2, with A2-165 as the representative strain[2]. However, it has remained elusive yet where these two phylogroups are localized in the feces and more specifically, what their location is towards the epithelial cells. Therefore we performed this study on stools of 12 healthy volunteers.
Materials and Methods: The simple but yet practical straw technique[1] was used to punch fecal stools while keeping the structure preserved. This was followed by 4% PFA fixation and fluorescence *in situ* hybridization (FISH) with new specific probes designed for these two phylogroups based on their 16s rRNA. Epi-fluorescence microscopy was performed to localize the hybridized bacteria.
Results: The performed study demonstrated that phylogroup 2 of *F. prausnitzii* is more dominant next to the mucus layer in comparison with phylogroup 1, which means it colonizes the feces/mucus interphase . In addition, FISH showed that *F. prausnitzii* is forming biofilms around some food particles in the luminal part of the gut.
Conclusion: Phylogroup 2 of *F. prausnitzii* is more attracted to the mucosal side of the stool in comparison by phylogroup 1, most likely because it is more capable of benefiting from the oxygen that diffuses in the lumen through the epithelial cells for shuttling electrons away from the bacteria. We observed that particular food particles have abilities to support the growth of both phylogroups of *F. prausnitzii* in the gut where they form biofilms and micro-colonies. Which of the two phylogroups has most health beneficial effects on the host remains to be investigated.

1- Swidsinski A et al., 2010. Gastroenterol Clin Biol.34 Suppl 1:S79-92.
2- Lopez-slies et al., 2012. Appl Environ Microbiol.78:420-428
3- Khan et al., 2012.Antioxid Redox Signal.15;17(10):1433-40.

SIV5-FG

Actinobacteria as essential nutritional symbionts in pyrrhocorid bugs

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Actinobacteria have recently been found to engage in a range of defensive symbioses with insects, but reports of nutritional contributions to their hosts have been exceptionally rare. Red firebugs (*Pyrrhocoris apterus*, Hemiptera, Pyrrhocoridae) are known to harbor the actinobacterial symbiont *Coriobacterium glomerans* in the M3 region of their mid-gut, but their functional importance remained enigmatic. We used deep-sequencing of bacterial 16S rRNA amplicons to comprehensively characterize the M3 microbial community of *P. apterus* as well as 20 other species of pyrrhocorid bugs (Sudakaran et al. in press). Interestingly, a characteristic microbiota consisting mainly of Actinobacteria (*C. glomerans* and *Gordonibacter* sp.), Firmicutes (*Clostridium* sp. and *Lactococcus* sp.), and Proteobacteria (*Klebsiella* sp. and an unknown Rickettsiales bacterium) was highly conserved across pyrrhocorid bugs, indicating a stable association that may result from host-microbiota co-evolution. Experimental elimination of symbionts in two host species (*P. apterus* and *Dysdercus fasciatus*) resulted in significantly higher mortality and reduced growth rates, demonstrating that the microbial community plays an important role for host nutrition. Fitness of aposymbiotic bugs could be completely restored by re-infection with the original microbiota, while reciprocal cross-infections of microbial communities across the two pyrrhocorid host species only partially rescued fitness (Salem et al. in press). Exhaustive community-level analyses of the experimentally manipulated bugs by quantitative PCRs targeting the six dominant bacterial strains allowed us to link the observed fitness effects to the abundance of the two actinobacterial symbionts. Further experiments provided evidence that these bacteria supplement limiting nutrients or aid in the degradation of complex dietary compounds. These findings expand our understanding of actinobacterial associations with insects and shed light on a nutritional mutualism that has likely contributed to the ecological success of the economically important group of pyrrhocorid bugs.

Salem, H., Kreutzer, E., Sudakaran, S. & Kaltenpoth, M. in press. Actinobacteria as essential symbionts in firebugs and cotton stainers (Hemiptera, Pyrrhocoridae). *Environ. Microbiol.*
Sudakaran, S., Salem, H., Kost, C. & Kaltenpoth, M. in press. Geographic and ecological stability of the symbiotic mid-gut microbiota in European firebugs, *Pyrrhocoris apterus* (Hemiptera; Pyrrhocoridae). *Mol. Ecol.*

SIV6-FG

Exploring *Bifidobacterium bifidum* S17 for potential players in host-microbe interactions by genomic and proteomic approaches

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Bifidobacteria are an important bacterial component of the human intestinal microbiota and are frequently used as probiotics due to the various health-promoting effects thatbare associated with their presence in the human lower gastrointestinal tract. *Bifidobacterium bifidum* S17 is a promising candidate able to tightly adhere to intestinal epithelial cells (IEC) and showing potent anti-inflammatory activity in vitro and effect in several murine models of chronic intestinal inflammation.
To further elucidate the mechanisms contributing to host-colonization and inhibition of inflammation by B. bifidum S17, we have sequenced and annotated the genome of this strain. Furthermore, 2D total proteome maps were established for bacteria grown in vitro. Several surface proteins, including the *B. bifidum*-specific adhesin BopA, were identified in the genome and shown to be expressed *in vitro*, with higher expression during exponential growth phase. This correlated with increased adhesion of *B. bifidum* S17 to Caco-2 cells in exponential phase. *B. bifidum* S17 possesses four putative pili-encoding gene clusters, which are expressed *in vitro* as well. Moreover, scanning and transmission electron microscopy images provided evidence for the presence acidic polysaccharides on the bacterial cell surface.
Furthermore, the effect of *B. bifidum* S17 on various subsets of $CD4^+$ T-cells and the cytokine milieu in the intestinal mucosa was investigated in two models of colitis (DSS-induced colitis and T-cell transfer model in $Rag^{-/-}$ mice). The results indicate that reduction of clinical symptoms of colitis upon pre-treatment of mice with *B. bifidum* S17 is associated with a reduction in tissue levels of pro-inflammatory cytokines (TNF-a, IL-6, IFN-g), reduced numbers of total CD4+ T cells and increased frequencies of $FoxP3^+$ regulatory T cells.

SIV7-FG

Thaumarchaeota on human skin - implications for medical surveys?

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Archaea, the third domain of life, were for long time thought to be an ancient form of microorganisms that populate only extreme environments. Recently, mesophilic archaea (Thaumarchaeota) were found to be major contributors of the nitrogen cycle in terrestrial soils and the sea but were also found in man-made environments like clean room facilities. We expanded the exploration of archaeal biotopes to intensive care units of two hospitals that are strongly influenced by the human microbiome and were able to detect the same archaeal group in samples taken from floors. Consequently, we investigated the presence of Archaea in the human skin microbiome by screening 13 different human subjects and report for the first time the successful quantification and visualization of Archaea on human skin. Noteworthy, Archaea comprised up to 4.2% of the entire genetic prokaryotic skin microbiome. Most of the gene signatures analyzed belonged to phylum Thaumarchaeota, which have so far gone undetected in human microbiome surveys. Based on our results we suggest an important role of Archaea on human skin and hypothesize their involvement in stabilization of the natural protective layer.

SIP1-FG

Physicochemical gut conditions and intestinal microbiota of wood-feeding cockroaches (Blaberidae: Panesthiinae)

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The symbiotic digestion of cellulose in the guts of termites has made them favorite research objects of microbiologists. However, little is known about the gut microbiota of the distantly related wood-feeding cockroaches in the family Blaberidae and its role in digestion. Here, we investigated physicochemical gut conditions, microbial metabolites, and bacterial community structure in the different gut compartments of *Panesthia angustipennis* and *Salganea esakii* (both Panesthiinae), using a combination of microsensor techniques and cloning and pyrotag analysis of 16S rRNA genes. As in omnivorous cockroaches (1), the gut of *P. angustipennis* showed a slightly acidic to neutral pH and a negative redox potential. This is in agreement with the accumulation of hydrogen in the crop and midgut, and a strongly hydrogen-limited methanogenesis in the hindgut paunch. Cellulase activities and high glucose concentrations were present in extracts of all compartments, with acetate, lactate, and other short-chain fatty acids as microbial fermentation products. The gut microbiota of both *P. angustipennis* and *S. esakii* differed strongly between compartments, with highest densities in the hindgut. There were high similarities to omnivorous cockroaches and strong differences to wood-feeding termites and *Cryptocercus punctulatus*. However, there were also many lineages that were common to all groups, particularly among the Bacteroidetes and Clostridiales. Comparative analysis of bacterial 16S rRNA gene sequences obtained from clone libraries of hindgut contents provided numerous evidence for co-cladogenesis, suggesting that the bacterial lineages in cockroaches and termites trace back to a common evolutionary ancestor.

(1) C. Schauer, C.L. Thompson, and A. Brune, *Appl. Environ. Microbiol.* 78, 2758-2767 (2012).

Personalia aus der Mikrobiologie 2012

Habilitationen

Dörte Becher habilitierte sich am 27. Januar 2012 an der Universität Greifswald (Towards the entire proteome: novel qualitative and quantitative approaches for global proteome analyses in gram positive bacteria).

Gunther Döhlemann habilitierte sich am 16. Mai 2012 an der Universität Marburg (Etablierung der kompatiblen Interaktion des Brandpilzes *Ustilago maydis* mit seiner Wirtspflanze Mais).

Karl-Tilmann Weber habilitierte sich am 16. Juni 2012 an der Universität Tübingen (Genombasierte Ansätze zur Analyse der Kirromycin-Biosynthese und weiterer Antibiotika-Biosynthesewege).

Ralph Bertram habilitierte sich am 18. Juni 2012 an der Universität Tübingen (Etablierung und Anwendung neuer Rekombinations- und Induktionssysteme zur Charakterisierung von Dormanz und Kohlenstoffmetabolismus in Firmicutes)

Peter Staib habilitierte sich am 15. Oktober 2012 an der Universität Jena (Molekularbiologische Untersuchungen zur Biologie und Pathogenität von *Candida albicans* und Dermatophyten).

Jens Glaeser habilitierte sich am 5. November 2012 an der Universität Gießen (Effects of photosensitized singlet oxygen formation on bacteria).

Kai Martin Thormann habilitierte sich am 19. Dezember 2012 an der Universität Marburg (NO! – nitric oxide signaling in bacteria).

Ruf angenommen

Beatrix Süß von der Universität Frankfurt/Main übernahm am 1. April 2012 die W3-Professur Synthetic Genetic Circuits an der Technischen Universität Darmstadt.

Peter Graumann von der Universität Freiburg übernahm am 1. April 2012 die W3 Professur für den Lehrstuhl Biochemie an der Universität Marburg.

Marc Bramkamp von der Universität zu Köln übernahm am 1. Mai 2012 die W2-Professur für Mikrobiologie an der Ludwig-Maximilians-Universität München.

Sven Krappmann von der Universität Würzburg übernahm am 1. Mai 2012 eine W2-Professur für Klinische Mikrobiologie und Immunologie an der Universität Erlangen-Nürnberg.

Alexander Böhm-Bettenworth von der Universität Würzburg übernahm am 1. Juli 2012 eine W2-Professur am LOEWE-Zentrum für Synthetische Mikrobiologie an der Universität Marburg.

Annegret Wilde von der Universität Gießen übernahm am 1. August 2012 eine W3-Professur am Institut für Biologie III der Universität Freiburg.

Heide Schulz-Vogt vom Max-Planck-Institut für Marine Mikrobiologie in Bremen übernahm am 1. September 2012 die W3-Professur für den Lehrstuhl Biologische Meereskunde an der Universität Rostock, verbunden mit der Leitung der Sektion Biologische Meereskunde am Leibniz-Institut für Ostseeforschung Warnemünde.

Matthias Boll von der Universität Leipzig übernahm am 21. September 2012 die W3-Professur für Mikrobiologie an der Universität Freiburg.

Julia Frunzke vom Institut für Bio- und Geowissenschaften, IBG-1: Biotechnologie am Forschungszentrum Jülich hat am 28. November 2012 die W1-Juniorprofessur für das Fach „Signaltransduktion und Populationsheterogenität in industriellen Mikroorganismen“ an der Universität Düsseldorf übernommen.

Emeritierungen/ Pensionierungen

Manfred Kröger vom Institut für Mikrobiologie und Molekularbiologie an der Universität Gießen wurde am 31. März 2012 pensioniert.

Hans-Peter Fiedler vom Interfakultären Institut für Mikrobiologie und Infektionsmedizin an der Universität Tübingen wurde am 1. August 2012 pensioniert.

Rüdiger Bode vom Institut für Mikrobiologie der Universität Greifswald wurde am 30. September 2012 pensioniert.

Wissenschaftliche Preise

Regine Kahmann und **Rudolf K. Thauer** vom Max-Planck-Institut für terrestrische Mikrobiologie in Marburg wurden am 2. Februar 2012 als Fellow in die American Academy of Microbiology aufgenommen.

Katja Seider vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut in Jena – erhielt am 18. Februar 2012 den Forschungspreis der DGHM-Fachgruppe Eukaryontische Krankheitserreger für ihre Arbeiten zur Infektionsbiologie von *Candida albicans*.

Ewa Maria Musiol von der Universität Tübingen erhielt am 23. Februar 2012 den DECHEMA-Biotechnologie-Doktoranden-Preis für Naturstoff-Forschung 2012 für ihre Arbeiten über „Ethylmalonyl-CoA Spezifität der Acyltransferase KirCII aus dem Kirromycin-Biosynthesegencluster“.

Fabian Horn vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut in Jena – erhielt am 13. März 2012 in Jena den „Presentation award des 5th ILRS Symposiums“ für seine Arbeiten über die Systembiologie von mikrobiellen Interaktionen.

Sonja-Verena Albers vom Max-Planck-Institut für terrestrische Mikrobiologie erhielt am 19. März 2012 den VAAM-Forschungspreis für ihre Arbeiten über die Oberflächenstrukturen von Archaeen.

Bork Berghoff (Uni Gießen), **Eva Biegel** (Uni Frankfurt), **Cornelia Welte** (Uni Bonn) und **Frank Uliczka** (TU Braunschweig) erhielten am 20. März 2012 den VAAM-Promotionspreis.

Qian Chen vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut in Jena – erhielt am 24. März 2012 den Else-Körner-Fresenius-Preis für ihre Arbeiten über „A novel hybrid CFHR2/CFHR5 gene in membranoproliferative glomerulonephritis type II“.

Antje Boetius vom Max-Planck-Institut für Marine Mikrobiologie in Bremen und Alfred-Wegener-Institut für Polar- und Meeresforschung in Bremerhaven erhielt Anfang Juni 2012 den KIT-Preis Heinrich-Hertz-Gastprofessorin 2012 der Karlsruher Universitätsgesellschaft (KUG) und des Karlsruher Instituts für Technologie (KIT).

Nicole Dubilier erhält renommierten Wissenschaftspreis

Prof. Dr. Nicole Dubilier (Max-Planck-Institut für Marine Mikrobiologie und MARUM Universität Bremen) wurde im Dezember 2012 für das Wissenschafts-Förderprogramm der Marine-Mikrobiologie-Initiative der Gordon- und Betty-Moore-Stiftung ausgewählt. Als einzige der 16 ausgezeichneten Wissenschaftler arbeitet sie nicht an einem Forschungsinstitut in den USA. Mit 200 000 bis 500 000 US-Dollar pro Jahr und Wissenschaftler fördert die Stiftung Pionierprojekte der marinen mikrobiellen Ökologie.

Nicole Dubilier leitet die Symbiose-Gruppe am Max-Planck-Institut für Marine Mikrobiologie und ist Professorin im Fachbereich Biologie/Chemie der Universität Bremen. Ihre Forschungsthemen sind die Symbiosen zwischen Meerestieren und Bakterien, die aus exotischen Habitaten wie den Hydrothermalquellen der Tiefsee, aber auch aus leichter zugänglichen Untersuchungsgebieten wie den Seegraswiesen im Mittelmeer stammen. 2011 zeigten sie und ihre Kollegen in der Fachzeitschrift Nature, dass bestimmte Tiefseemuscheln über eine Art Brennstoffzelle in Form von symbiontischen Bakterien verfügen, die Wasserstoff direkt als Energiequelle nutzen (s. a. BIOspektrum 1/10, S. 12, 6/06, S. 600). Dubilier möchte mit dem Forschungsgeld "neue Ansätze verwirklichen, die zwar etwas riskant, aber sehr vielversprechend sind. Symbiosen spielen eine Schlüsselrolle für das marine Leben und die Biodiversität, besonders dort, wo es knapp um die Ressourcen steht, wie zum Beispiel die Tiefsee."

Die Moore-Stiftung will den Forschern vor allem Freiheiten und die Flexibilität geben, um nicht von Vorschriften und Regeln behindert zu werden. Das Preisgeld soll ihnen mehr Risikobereitschaft für neue Kooperationen ermöglichen. „Diese Forscher sollen unsere bisherigen Ansichten über den Lebensraum Ozean radikal in Frage stellen", so Vicki Chandler, die das Forschungsprogramm leitet. „Die marinen Mikroorganismen stellen mehr als 90 Prozent der Biomasse in den Meeren dar und beeinflussen den Zustand der Ozeane wesentlich. Bisher wissen wir nur, wer sie sind, aber nicht, was sie machen und wie sie interagieren. Mit unserer finanziellen Unterstützung wollen wir Wissenschaftler aus verschiedenen Disziplinen zusammen bringen und die Wissenslücken schließen."

(stö)

Daniel Lanver vom Max-Planck-Institut für terrestrische Mikrobiologie in Marburg erhielt am 13. Juni 2012 die Otto-Hahn-Medaille für seine Arbeiten über die „Perzeption hydrophober Oberflächen durch den pflanzenpathogenen Pilz *Ustilago maydis".*

Michael Hecker von der Universität Greifswald erhielt am 30. Juni 2012 den Robert-Pfleger-Forschungspreis für seine Arbeiten in der Proteomik und Infektionsbiologie.

Oleksandra Fokina von der Universität Tübingen erhielt am 21. Juli 2012 den Promotionspreis der Universität Tübingen und am 19. November den Promotionspreis der Reinhold-und-Maria-Teufel-Stiftung Tuttlingen für ihre Arbeiten über „From Metabolite Sensing to Protein Regulation by the Synechococcus elongatus PCC 7942 PII Protein".

Sebastian Suerbaum von der Medizinischen Hochschule Hannover, Institut für Medizinische Mikrobiologie und Krankenhaushygiene, erhielt am 7. September 2012 den Heinz P. R. Seeliger-Preis.

Garabed Antranikian von der Technischen Universität Hamburg-Harburg erhielt am 13. September 2012 in Sevilla den Life Time Achievement Award der International Society for Extremophiles.

Martin Kreutzer vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut in Jena – erhielt am 29. September 2012 in Braunschweig den „Best lecture award" des VAAM-Workshops Biologie und Chemie von Antibiotika-produzierenden Bakterien und Pilzen.

Qian Chen vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut in Jena – erhielt am 15. Oktober 2012 den „Best lecture award des XXIV International Complement Workshop", Kreta, für ihre Arbeiten über „A novel hybrid CFHR2/CFHR5 gene in membranoproliferative glomerulonephritis type II".

Teresia Hallström vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut in Jena – erhielt am 15. Oktober 2012 den Best lecture award des XXIV International Complement Workshop, Kreta, für ihre Arbeiten über „Microbial pathogens of diverse origin inhibit the terminal complement pathway: A common immune evasion strategy?".

Pranatchareeya Chankhamjon, Thorsten Heinekamp, Martin Roth, Daniel Scharf und **Kirstin Scherlach** vom Leibniz-Institut für Naturstoff-Forschung und Infektionsbiologie – Hans-Knöll-Institut in Jena – erhielten am 5. November 2012 medac-Forschungspreis 2012 für ihre Arbeiten zur mikrobiellen Naturstoff-Biosynthese.

Stefan Häusler vom Max-Planck-Institut für Marine Mikrobiologie, Bremen erhielt Mitte November 2012 den Preis für die beste Vortragspräsentation auf dem Symposium „Israel Society for Microbiology, Fall meeting, 2012 für seinen Vortrag zum Thema "Rich microbial communities in and around a complex system of underwater springs in the Dead Sea".

Ewa Maria Musiol, Dorothee Kretschmer, **Martin Schlag** und **Christoph Ernst** von der Universität Tübingen erhielten am 19. November 2012 den Promotionspreis der Reinhold-und-Maria-Teufel-Stiftung Tuttlingen.

Promotionen 2012

Universität Bayreuth

Bach Hue Nguyen: Identification of substrate proteins of FtsH during sporulation of *Bacillus subtilis*
Betreuer: Wolfgang Schumann

Humboldt-Universität Berlin

Johannes Fritsch: Molekulare Grundlagen der Sauerstofftoleranz der membrangebundenen [NiFe]-Hydrogenase aus *Ralstonia eutropha* H16
Betreuerin: Bärbel Friedrich

Freie Universität Berlin

Natalia Tschowri: Signaltransduktion in einem Blaulicht/EAL-Domänen-gesteuerten Genregulationsmechanismus in *Escherichia coli*
Betreuerin: Regine Hengge

Nicole Sommerfeldt-Impe: Regulation von Biofilmfunktionen durch c-di-GMP in *Escherichia coli*
Betreuerin: Regine Hengge

Noel Molière: The role of *Bacillus subtilis* Clp/Hsp100 Proteases in the regulation of swimming motility and stress response
Betreuer: Kürsad Turgay

Externe Promotionen

Christof Junkes: Strukturelle Grundlagen der antimikrobiellen Aktivität und Selektivität kleiner cyclischer Peptide
Betreuer: Michael Bienert (Leibniz-Institut für Molekulare Pharmakologie), Rupert Mutzel

Skadi Kull: Massenspektrometrische Verfahren zur simultanen Detektion von pflanzlichen und mikrobiellen Toxinen
Betreuer: Dieter Nauman (Robert Koch-Institut), Rupert Mutzel

Faisal Ashgar Khattak: An efficient method for random mutagenesis in *Mycobacterium avium* subsp. *hominissuis* and for screening of mutants affected in virulence
Betreuer: Reinhard Burger (Robert Koch-Institut), Rupert Mutzel

Tim Mak: Host modulating properties of *Propionibacterium acnes*
Betreuer: Thomas Meyer (MPI Infektionsbiologie), Rupert Mutzel

Christian Linke: Identification of novel mechanisms controlling cell cycle progression in S. *cerevisiae*
Betreuer: Hans Lehrach (MPI MolGen), Rupert Mutzel

Alexander Falenski: Tenaziät von hochpathogenen Erregern in Lebensmitteln und Schnellmethoden zu deren Nachweis am Beispiel *Brucella*
Betreuer: Bernd Appel (BfR), Rupert Mutzel

Shahjahan Shaid: Development of an Antibody-Based Differentiation of *Francisella tularensis* Subspecies
Betreuer: Roland Grunow (Robert Koch-Institut), Rupert Mutzel

Universität Bielefeld

Hanna Bednarz: Etablierung einer Transposon-basierten Methode zur fluoreszenten Markierung und mikroskopischen Aufklärung subzellulärer Proteinlokalisierungen in *S. meliloti* Rm1021
Betreuer: Karsten Niehaus

Olga Blifernez-Klassen: Molecular mechanisms behind the adjustment of phototrophic light-harvesting and mixotrophic utilization of cellulosic carbon sources in *Chlamydomonas reinhardtii*
Betreuer: Olaf Kruse

Nicole Günther: Adaptation von *P. aeruginosa* an die Mukoviszidose-Lunge: Charakterisierung des kleinen Hitzeschockproteins IbpA
Betreuer: Karsten Niehaus

Kai-Hendrik Lerche: Stable nuclear transformation of the volvocine algae *Gonium pectorale, Pandorina morum* and *Eudorina elegans*
Betreuer: Armin Hallmann

Kerstin Mayer: Die extrazellulären Serinproteasen von *Clavibacter michiganensis* subsp. *michiganensis* – Versuche zur Reinigung, Aktivität und ihrer Funktion
Betreuer: Rudolf Eichenlaub

Yaarub R. Musa: Adaptation of the proteome and phosphoproteome of *Xanthomonas campestri*s pv. *campestris* B100 to the different growth phases during the growth under batch culture conditions
Betreuer: Karsten Niehaus

Jens Schneider: Engineering *Corynebacterium glutamicum* for production of putrescine,
Betreuer: Volker F. Wendisch

Jessica Schneider: Sequenzanalyse, Annotation und Stoffwechselrekonstruktion der biotechnologisch relevanten Hefen *Wickerhamomyces ciferrii* und *Wickerhamomyces anomalus* mit der RAPYD-Plattform
Betreuer: Andreas Tauch

Jasmin Schröder: Genomic diversity of Corynebacteria: Insights into cheese ripening and the lifestyle of a human pathogen
Betreuer: Andreas Tauch

Helena Tews: Das Exoproteom von *Clavibacter michiganensis* subsp. *michiganensis* und Untersuchung von Mutanten im Sec- und Tat-Sekretionssystem
Betreuer: Rudolf Eichenlaub

Eva Trost: Das bakterielle Pangenom: Komparative Genomanalysen zur Identifizierung neuer Virulenzfaktoren des *Corynebacterium diphtheriae*-Clusters
Betreuer: Andreas Tauch

Tony Watt: Bioanalytical analysis of the carbon metabolism and type-III secretion system in *Xanthomonas campestris* pv. *campestris* under industrial-like conditions and in host mimicking environments
Betreuer: Karsten Niehaus

Universität Bochum

Vera Kock: Plastidäres RNA trans-Spleißen bei der Grünalge *Chlamydomonas reinhardtii*: Molekulargenetische und biochemische Analysen von RNA-Bindeproteinen
Betreuer: Ulrich Kück

Nadja Raatschen: Effects of ionophores and aminosterol antibiotics on the proteome of *Bacillus subtilis*
Betreuerin: Julia Bandow

Jan Gleichenhagen: Bakterielle Phospholipid N-Methyltransferasen: Charakterisierung enzymatischer Eigenschaften und Substratspezifitäten
Betreuer: Franz Narberhaus

Corinna Rademacher: Kupfer- und Tellurit-Toleranz in *Rhodobacter capsulatus*
Betreuer: Franz Narberhaus

Michael Schäkermann: Posttranslational regulation of LPS biosynthesis in *Escherichia coli* and other Gram-negative bacteria
Betreuer: Franz Narberhaus

Universität Bonn

Elisabeth Witt: Nebenreaktionen der Ectoin-Synthase aus *Halomonas elongata* DSM 2581^T und Entwicklung eines salzinduzierten Expressionssystems
Betreuer: Erwin A. Galinski

Susanne Schmitz: Vergleichende Untersuchungen zur Struktur und Expression von Antikörpern in *Nicotiana benthamiana* und CHO-Zellen zum Einsatz in der Krebstherapie
Betreuer: Uwe Deppenmeier

Frauke Grimm: Regulation des *dsr* Operons und Funktion der Proteine DsrR und DsrS im Schwefelpurpurbakterium *Allochromatium vinosum*
Betreuerin: Christiane Dahl

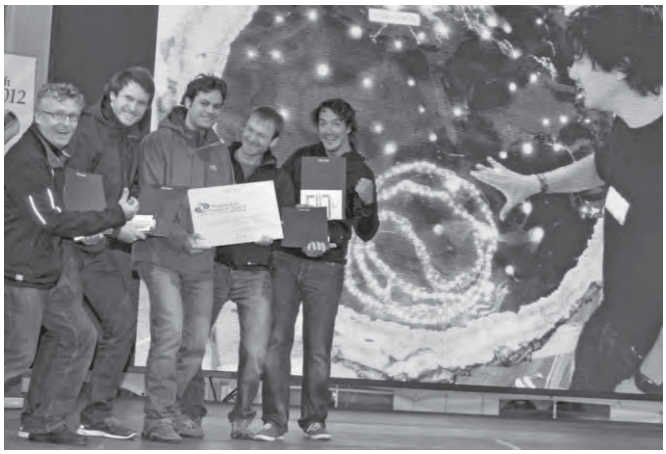
Verena Kallnik: Untersuchungen zur Polyol-Oxidation in *Gluconobacter oxydans* und *Thermotoga maritima*
Betreuer: Uwe Deppenmeier

Jasmin Dischinger: Novel lantibiotics from microbial genomes
Betreuer: Hans G. Sahl

Ahmed Gaballah: Molecular Analysis of the Chlamydial Anomaly: Role of the cytoskeleton protein MreB, the serine hydroxymethyl transferase GlyA and the penicillin binding proteins from *Chlamydophila pneumoniae*
Betreuer: Hans G. Sahl

Publikumspreis beim Wissenschaftssommer in Lübeck

Eine Reise in die Welt der Mikroorganismen



So sehen Gewinner aus: Manfred Schlösser, Andreas Krupke, Wolfgang Hankeln, Lorenzo Franceschinis und Dennis Fink (von links) vor einem millionenfach vergrößerten Bakterium im Querschnitt.

Ein Team des Bremer Max-Planck-Instituts für marine Mikrobiologie hat den mit 10 000 Euro dotierten Preis „Wissenschaft interaktiv 2012" gewonnen, den die Besucher des Wissenschaftssommers jedes Jahr vergeben. Das ausgezeichnete Exponat führte die Besucher in die Welt der Mikroorganismen: In welcher Menge kommen sie in der Umwelt vor? Welche chemischen Verbindungen können sie zum Leben nutzen? Welche Rolle spielen sie im Klimawandel? Ein Video zeigt die Entstehung des Ausstellungsstücks: ww.youtube.com/user/MediomixMedia.

Mit einem überdimensionalen Modell einer Bakterienzelle, mit Videos und Filmclips über besondere Arten wie die riesige Schwefelperle (*Thiomargarita namibiensis*) führten die Gewinner in die Welt der Mikroorganismen. Wer wollte, konnte mithilfe seines Smartphones noch tiefer in die Materie einsteigen. Wie immer beim Wissenschaftssommer stand jedoch der direkte Dialog zwischen Forschern und Besuchern im Vordergrund: „Es ist sehr wichtig, dass wir lernen, die Wissenschaft, die uns begeistert, auch zu kommunizieren. Uns hat es Riesenspaß gemacht", sagte Dennis Fink bei der Preisverleihung.

Fink und sein Kollege Wolfgang Hankeln, Mitglieder des Erfolgsteams, sehen ihre berufliche Zukunft nach der Promotion am Bremer Max-Planck-Institut in der Wissenschaftskommunikation. Ihr Konzept für eine Agentur, die Forscher bei der Vermittlung ihrer Themen in der Öffentlichkeit unterstützen soll, ist dem Bundesministerium für Wirtschaft und Technologie und der EU sogar ein EXIST-Gründerstipendium wert.

(mpg)

Miriam Wilmes: Antibiotic mechanisms of invertebrate and mammalian defensins,
Betreuer: Hans G. Sahl

Technische Universität Braunschweig

Mareike Jogler: Niche adaptation and microdiversity among populations of planktonic bacteria
Betreuer: Jörg Overmann, DSMZ

Simone Karrie: Incorporation of the prosthetic heme group into cytoplasmic and membrane proteins
Betreuer: Dieter Jahn

Isam Haddad: Entwicklung informatischer, mathematischer und instrumenteller Methoden zur Analyse physiologischer Prozesse in mikrobiellen Populationen
Betreuer: Dieter Jahn

Universität Bremen/MPI für Marine Mikrobiologie Bremen

Kirsten Webner: Die Gene der (1-Methylalkyl)Succinat-Synthase im anaeroben *n*-Alkanabbau des Betaproteobakteriums Stamm HxN1
Betreuer: Friedrich Widdel

Olivera Svitlica: Studies on aerobic ammonia oxidation and denitrification in stratified habitats
Betreuer: Rudolf Amann

Chia-I Huang: Molecular Ecology of Free-Living Chemoautotrophic Microbial Communities at a Shallow-sea Hydrothermal Vent
Betreuer: Rudolf Amann

Vladimir Bondarev: Physiology of *Pseudovibrio sp. FO-BEG1* – a facultatively oligotrophic and metabolically versatile bacterium
Betreuerin: Heide Schulz-Vogt

Sara Kleindienst: Hydrocarbon-degrading sulphate-reducing bacteria in marine hydrocarbon seep sediments
Betreuer: Rudolf Amann

Dennis Enning: Studies on aerobic ammonia oxidation and denitrification in stratified habitats
Betreuer: Friedrich Widdel

Petra Pop Ristova: Deep Sea Oases of Life: Insights from Cold Seep and Wood Fall Ecosystems
Betreuerin: Antje Boetius

Cecilia Wentrup: Acquisition and activity of bacterial symbionts in marine invertebrates
Betreuerin: Nicole Dubilier

Manuel Kleiner: Metabolism and evolutionary ecology of chemosynthetic symbionts from marine invertebrates
Betreuerin: Nicole Dubilier

Johannes Zedelius: Anaerobic *n*-alkane degradation by Gammaproteobacterium strain HdN1 coupled to nitrate respiration
Betreuer: Friedrich Widdel

Abdul Rahiman Sheik: Viral regulation on nutrient assimilation by algae and prokaryotes
Betreuer: Marcel Kuypers

Tim Kalvelage: Nitrogen losses and nutrient regeneration in oxygen minimum zones
Betreuer: Marcel Kuypers

Ines Heisterkamp: Microbial nitrous oxide production and nitrogen cycling associated with aquatic invertebrates
Betreuer: Bo Barker Jørgensen

Martin Glas: The importance of microgradients for marine calcifiers
Betreuer: Dirk de Beer

Judith Neumann: Effect of high CO_2 and low pH on benthic communities of the deep sea
Betreuerin: Antje Boetius

International Jacobs University Bremen

Anna Klindworth: RNA based research – development, application and analysis within the MIMAS project
Betreuer: Frank-Oliver Glöckner

Marc Weber: Capturing Biodiversity in Metagenomic Data – Design, Implementation and Evaluation of a Bioinformatic Method for Binning and Classification of DNA Sequences
Betreuer: Frank-Oliver Glöckner

Technische Universität Darmstadt

Larissa Marschaus: Analyse einer divergenten Promotorregion in *Halobacterium salinarum*: Wirkung einzelner Promotorelemente und Stärke der Aktivierung
Betreuerin: Felicitas Pfeifer

Technische Universität Dortmund

Daniel Kuhn: Systems biotechnology for sustainable biocatalytic production of (*S*)-styrene oxide
Betreuer: Bruno Bühler, Andreas Schmid

Rohan Karande: Development and application of microreactors for biocatalytic reactions,
Betreuer: Katja Bühler, Andreas Schmid

Babu Halan: Biofilms as living catalysts for fine chemical synthesis: analysis, process design, and scale up
Betreuer: Katja Bühler, Andreas Schmid

Technische Universität Dresden

Patrick Richthammer: Molekulare Mechanismen der Bildung der nanostrukturierten Silicatschalen von Diatomeen
Betreuer: Karl-Heinz van Pée

Patrick Petzsch: Generierung eines funktionellen Kataloges für das Genom der Hefe *Yarrowia lipolytica* und dessen Anwendung bei Microarray-Untersuchungen zur Genexpression
Betreuer: Gerold Barth

Universität Düsseldorf/ Forschungszentrum Jülich

Sandra Scheele: Untersuchungen zur sekretorischen Proteingewinnung industriell relevanter Enzyme mit *Corynebacterium glutamicum*
Betreuer: Roland Freudl

Ava Rebecca Chattopadhyay: Regulatorische Aspekte der Expression und Sekretion heterologer Proteine in *Corynebacterium glutamicum*
Betreuer: Roland Freudl

Stefan Fleckenstein: Untersuchungen zur Membranintegrität während der Tat-abhängigen Proteintranslokation in *Escherichia coli*
Betreuer: Roland Freudl

Andreas Otten: Metabolic Engineering von *Corynebacterium glutamicum* für die Produktion einer Dicarbonsäure
Betreuer: Michael Bott

Solvej Siedler: Increasing the NADPH supply for whole-cell biotransformation and development of a novel biosensor
Betreuer: Michael Bott

Janine Richhardt: *Gluconobacter oxydans* strain development: studies on central carbon metabolism and respiration
Betreuer: Michael Bott

Universität Erlangen-Nürnberg

Lisa Ott: Interaction of *Corynebacterium diphtheriae* with host cells
Betreuer: Andreas Burkovski

Anna Srebrzynski: Differentielle Regulation p65-abhängiger Systeme
Betreuer: Andreas Burkovski

Nadine Steinert: Quantitative und qualitative Analyse der Transkription CcpA-regulierter Gene
Betreuer: Andreas Burkovski

Christoph Stöckle: TIP-vermittelte Induktion Tetrazyklin-abhängiger Transregulatoren in *Saccharomyces cerevisiae* und HeLa-Zellen
Betreuer: Andreas Burkovski

Andrea Wünsche: Identification and characterization of novel interaction partners of CcpA in *Bacillus subtilis*
Betreuer: Andreas Burkovski

Universität Frankfurt am Main

Thorsten Subtil: Aufnahme und Co-Verstoffwechselung von Pentosen mit Glukose in *Saccharomyces cerevisiae*
Betreuer: Eckhard Boles

Christoph Schorsch: Molekulargenetische Optimierung der Sphingoidbasen-Produktion der nicht-konventionellen Hefe *Pichia ciferrii*
Betreuer: Eckhard Boles

Janin Burkhardt: Der DNA-Translokator in *Thermus thermophilus* HB27: Strukturelle und funktionelle Charakterisierungen des Sekretinkomplexes PilQ
Betreuerin: Beate Averhoff

Universität Freiburg

Felix Dempwolff: Self assembly of cytoskeletal elements and the role of membrane proteins on cell shape and division
Betreuer: Peter Graumann

Marc Eisemann: Characterization of DnaA mutant proteins from *Bacillus subtilis* Novel insights into the initiation of replication
Betreuer: Peter Graumann

Universität Gießen

Sebastian Frühwirth: Der Blaulichtrezeptor Cryptochrom B aus *Rhodobacter sphaeroides* und dessen Einfluss auf licht- und sauerstoffabhängige Regulationswege
Betreuerin: Gabriele Klug

Verena Peuser: Regulatory links between metal metabolism and oxidative stress response in *Rhodobacter sphaeroides*
Betreuerin: Gabriele Klug

Thomas Wallner: Funktionelle Analyse konservierter Gene aus Cyanobakterien und Chloroplasten: Untersuchungen zur Funktion und physiologischen Relevanz der *Synechocystis* sp. PCC6803-Gene *ycf34*, *ycf54* und *ycf59*
Betreuerin: Annegret Wilde

Tom Rische: Untersuchungen zur ribonukleolytischen Aktivität und zur Funktion der RNase J aus *Rhodobacter sphaeroides*
Betreuerin: Gabriele Klug

Stefanie P. Glaeser: Singlet Oxygen – a natural stress factor – shaping the composition of heterotrophic bacterioplancton communities in surface waters of the humic lake Große Fuchskuhle
Betreuer: Peter Kämpfer

Universität Göttingen

Oliver Voigt: The influence of autophagy on the fruiting-body development of the filamentous ascomycete *Sordaria macrospora*
Betreuerin: Stefanie Pöggeler

Doris Petroi: Alpha-synuclein in *Saccharomyces cerevisiae*: model for aggregate clearance, cell survival, and influence of autophagy
Betreuer: Gerhard Braus

Jennifer Gerke: Secondary metabolism and development in the filamentous fungus *Aspergillus nidulans*: activation of silent gene clusters and characterization of the SAM synthetase SasA
Betreuer: Gerhard Braus

Martin Christmann: Deneddylation and fungal development: regulation of Nedd8 protein modification by DenA and the COP9 signalosome
Betreuer: Gerhard Braus

Denise Lüttmann: Die regulatorischen Funktionen des paralogen Phosphotransferasesystems (PTS^{Ntr}) in *Escherichia coli*
Betreuer: Boris Görke

Fabian Rothe: Regulation der Aktivität und Lokalisation von Antiterminatorproteinen der BglG-Familie
Betreuer: Jörg Stülke

Universität Greifswald

Gustavo Gámez: Molecular analysis of the surface-exposed PavB protein and its two-component regulatory system on pneumococcal colonization and diseases
Betreuer: Sven Hammerschmidt

Mohammad Tauseef Asmat: Deciphering *Streptococcus pneumoniae* induced host cell signaling during internalization and calcium release from intracellular stores
Betreuer: Sven Hammerschmidt

Yvonne Jäschke: Funktionelle Analyse der Interaktion pleiotroper Faktoren der Histonmodifizierung mit spezifischen Regulatoren der Phospholipidbiosynthese in der Hefe *Saccharomyces cerevisiae*
Betreuer: Hans-Joachim Schüller

Judith Olzhausen: Genetische Analyse und metabolische Deregulation der Coenzym A-Biosynthese in der Hefe *Saccharomyces cerevisiae*
Betreuer: Hans-Joachim Schüller

Krishnatej Nishtala: Proteomic analysis of disease associated changes in animal models of dilated cardiomyopathy
Betreuer: Uwe Völker

Sven Bieler: Funktionelle Analyse der primären Stressantwort humaner Endothelzellen (HUVEC) auf Proteasomeninhibitoren im kardiovaskulären System
Betreuer: Uwe Völker

Theresa Winter: A physiological proteomic approach to address infection-related issues of Gram-positive bacteria
Betreuer: Michael Hecker

Katrin Kusch: Characterization and Regulation of Virulence Factors in *Staphylococcus aureus*
Betreuer: Michael Hecker

Stephan Michalik: Proteolysis at a proteome-wide scale in low GC, Gram-positive bacteria
Betreuer: Michael Hecker

Dierk-Christoph Pöther: Proceedings in the thiolome of low GC, Gram-positive bacteria
Betreuer: Michael Hecker

Alexander Reder: Integration of the sigmaB regulon into the regulatory network of *Bacillus subtilis*
Betreuer: Michael Hecker

Susanne Herter: Charakterisierung einer neuen Phenoloxidase von *Azotobacter chroococcum* und Anwendungsmöglichkeiten bakterieller und pilzlicher Phenoloxidasen für Aminierungsreaktionen an diphenolischen Substanzen
Betreuer: Frieder Schauer

Universität Halle-Wittenberg

Constanze Pinske: Reifung und Assemblierung der NiFe-Hydrogenasen aus *Escherichia coli*: Eisen und Regulation
Betreuer: Gary Sawers

Universität Hamburg

Jennifer Chow: Biochemical and Structural Characterization of Three Thermostable and Metagenome-Derived Lipolytic Enzymes
Betreuer: Wolfgang Streit

Arkadius Ozimek: Durchmusterung von Metagenombibliotheken nach biotechnologisch relevanten Phytasen und Entwicklung eines EGFP-basierten Nachweisverfahrens
Betreuer: Wolfgang Streit

Sandra Off: *Nitrospira* – Schlüsselorganismus der Nitritoxidation in gemäßigten und extremen Lebensräumen
Betreuerin: Eva Spieck

Technische Universität Hamburg-Harburg

Tobias Halbsguth: Flavin-abhängige Glukose-Dehydrogenasen für die Blutzuckerbestimmung
Betreuer: Garabed Antranikian

Medizinische Hochschule Hannover

Lucie Bartonickova: Characterisation of the virulence and colonisation factors T6SS and motility of *Helicobacter hepaticus*
Betreuerin: Christine Josenhans

Jenny Bode: Die Rolle des Komplementsystems bei der Pneumonie durch *Chlamydia psittaci* und andere Chlamydien
Betreuer: Andreas Klos

Lynn Kennemann: Genetic adaptation of *Helicobacter pylori* to the host individual
Betreuer: Sebastian Suerbaum

Elisabeth Wende: Functional effects of C5a receptor-like 2 (C5L2) co-expression with C5a receptor (C5aR) and The roles of C3a receptor (C3aR) and of C5L2 in a dextran sulfate-induced model of inflammatory bowel disease
Betreuer: Andreas Klos

Universität Hohenheim

Andrea Kroj: Untersuchungen zur spezifischen Genexpression von enterohämorrhagischen *Escherichia coli* (EHEC) in der Lebensmittelmatrix
Betreuer: Herbert Schmidt

Universität Jena

Jörg Linde: Systems Biology of human pathogenic fungi: Modeling transcriptional networks of virulence
Betreuer: Reinhard Guthke

Diana Barthel: Die Rolle von Plasminogen bei der Immunevasion der Gram-negativen Bakterien *Haemophilus influenzae* und *Borrelia burgdorferi*
Betreuer: Peter Zipfel

Hans-Wilhelm Nützmann: Protein acetyltransferases of *Aspergillus nidulans* – impact on secondary metabolism and fungal-bacterial interaction
Betreuer: Axel Brakhage

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Mikrobiologie

Dieses Lehrbuch basiert auf dem in den USA erfolgreichen Bestseller „Microbiology – An Evolving Science", der binnen zwei Jahren schon in 2. Auflage erschienen ist. Neues didaktisches Prinzip ist, dass das Grundlagenwissen der Mikrobiologie mit Beispielen aus der aktuellen Forschung erklärt wird. **Studierende der Biologie, Biotechnologie, Ingenieurwissenschaft und Medizin** erhalten auf diese Weise Zugang zur modernen Mikrobiologie, einer Wissenschaft mit Zukunft. Das Werk ist illustriert mit stilistisch einheitlichen und anschaulichen Farbgrafiken, die Erklärungen und korrekte Maßangaben enthalten. Spezielle Exkurse beschreiben die experimentellen Ansätze der mikrobiellen Spitzenforschung, Interviews mit prominenten deutschen Mikrobiologen geben der Wissenschaft ein menschliches Gesicht.

▶ Mikrobiologisches Praktikum

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A. Steinbüchel et al.

Mikrobiologisches Praktikum

In diesem Buch werden Mikroorganismen, ihre Wirkungen in Alltag und Umwelt sowie biotechnologische Produkte in einfachen und anschaulichen Versuchen sichtbar gemacht. Zu allen Versuchen werden die theoretischen Grundlagen ausführlich dargestellt. Zur Prüfungsvorbereitung und Nachbereitung dienen Fragen, die an jedes Kapitel anschließen. In der neuen Auflage sind alle Abbildungen in Farbe. Sie ist vollständig überarbeitet und um einige neue Versuche sowie zwei komplett neue Kapitel erweitert.
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